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DEVELOPMENT OF A NATURAL REARING SYSTEM TO IMPROVE SUPPLEMENTAL FISH QUALITY,

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DEVELOPMENT OF A NATURAL REARING SYSTEM TO IMPROVE SUPPLEMENTAL FISH QUALITY, 1991-1995

PROGRESS REPORT

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EXECUTIVE SUMMARY

In this report, the National Marine Fisheries Service (NMFS), in collaboration with the Bonneville Power Administration (BPA), the Washington State Department of Fish and Wildlife (WDFW), and the U.S. Fish and Wildlife Service (USFWS), presents research findings and guidelines for development and evaluation of innovative culture techniques to increase postrelease survival of hatchery fish. The Natural Rearing Enhancement System (NATURES) described in this report is a collection of experimental approaches designed to produce hatchery-reared chinook salmon (*Oncorhynchus tshawytscha*) that exhibit wild-like behavior, physiology; and morphology (see Section 1 for a description of experimental approaches). Our NATURES culture research for salmonids included multiple tests to develop techniques such as: raceways equipped with cover, structure, and natural substrates to promote development of proper body camouflage coloration; feed-delivery systems that condition fish to orient to the bottom rather than the surface of the rearing vessel; predator conditioning of fish to train them to avoid predators; and supplementing diets with natural live foods to improve foraging ability.

The underlying assumptions are that NATURES will: 1) promote the development of natural cryptic coloration and antipredator behavior; 2) increase postrelease foraging efficiency; 3) improve fish health and condition by alleviating chronic, artificial rearing habitat-induced stress; and 4) reduce potential genetic selection pressures induced by the conventional salmon culture environment. A goal in using NATURES is to provide quality fish for rebuilding depleted natural runs.

Unfortunately, most attempts to use hatchery-reared fish to rebuild naturally-spawning populations of Pacific salmon have yielded poor results. Although the protective nature of hatchery rearing increases egg-to-smolt survival, the postrelease survival of cultured salmonids is often considerably lower than that of wild-reared fish. Hatchery procedures may play a major role in the reduced performance of artificially-propagated fish.

In nature, stream dwelling Pacific salmon prefer solitary habitats that include small particle-size rock/gravel substrates and structure and overhead cover in the form of aquatic vegetation, fallen trees or undercut tree roots, and undercut banks. However, conventional salmonid hatchery practices are geared toward mass production under unnatural conditions. For example, fish are reared in the open, over uniform concrete substrate; provided no structures behind or under which to seek refuge from water current, predators, or dominant conspecifics; held at high, stress-producing densities; surface fed; and conditioned by surface feeding to approach large, moving objects at the surface.

These conventional fish culture practices are thought to induce domestication, reduce fitness of hatchery fish for subsistence in natural ecosystems, and, ultimately, lower smolt-to-adult survival compared to wild fish. It is probable that physiological stress and behavioral and morphological modifications, which result from this unnatural rearing environment, are major factors in the poor postrelease survival of many standard hatchery-reared salmon. The literature reviewed in Sections 2-3, describing wild/hatchery salmonid biology and fish culture techniques, suggests that juvenile postrelease survival of hatchery-reared Pacific salmon can be increased by modifying rearing environment.

Many of the differences that have been observed between cultured and wild fish are heavily influenced by the environment, and thus afford opportunity for remediation. Studies indicate that hatchery rearing environments can profoundly influence social behavior. For instance, food

availability and rearing densities in hatcheries typically far exceed those found in natural streams, and this may contribute to differences in agonistic behavior between hatchery- and wild-reared fish. In addition, cultured and naturally-reared salmonids respond differently to habitat, with wild fish utilizing both riffles and pools in streams and hatchery-reared fish primarily using pool environments that are similar to their raceway rearing experience.

Evidence also indicates that hatchery strains of salmonids have increased risk-taking behavior and lowered fright responses compared to wild fish, and are thus more vulnerable to predation. Surface feeding is known to condition hatchery fish to approach the surface of the water column, and this behavior can increase susceptibility to avian predation. Studies have also attributed increased avian and piscivorous predator vulnerability of hatchery fish to decreased crypsis for stream environments.

Hatchery rearing environments may also deprive salmon of the psychosensory stimuli necessary to fully develop antipredation behaviors. For example, some empirical evidence indicates that prior exposure to predation can improve subsequent predator avoidance ability for juvenile salmonids. Handling and transport inherent in hatchery operations also induces stress on juvenile salmonids, which may indirectly affect their vulnerability to predation through a reduction of fitness.

We believe that production of quality "wild-like" fish from hatcheries can be achieved through development of a NATURES system that minimizes husbandry induced differences between cultured fish and their wild-reared counterparts. A number of NATURES concepts have been explored, tested, and refined in Sections 4-12 of this report.

The WDFW Planning and Research Group (Olympia, Washington) identified fish marking and tagging procedures suitable for NATURES (Section 10). Identifying (i.e., marking) fish is an essential component of evaluating the effects of various NATURES rearing strategies. Marking methods best suited for NATURES studies are those which will not affect behavior, growth, locomotion, or survival, and meet other general requirements such as long-term retention and readability.

Mutilation and external tags are not acceptable marking methods for NATURES studies because of their adverse effects on behavioral and physiological factors. Branding techniques have been used in experiments for several decades and can provide a long-lasting external mark. Recent advancements in laser technology have improved the potential for laser marking as a viable tool; this mark may be more benign than branding and may last through the lifetime of some fish, particularly if methods can be developed that mark the soft fin rays.

Visual implant (V.I.) tags also show promise for use in NATURES studies. Injection of fluorescent materials have the advantage of being invisible until revealed by remote interrogation, thus eliminating observer bias and interactions between fish that might be associated with an externally visible tag. Panjet marks can also have a long retention time (i.e., several years) and can be used to mark young (30-40 mm) salmon fry.

Perhaps the two greatest concerns regarding all marking techniques are their degree of underwater visibility and their influence on fish behavior. Preliminary field evaluations of various marks were undertaken in 1992 to help establish protocols for evaluating these concerns. These observations indicated that laser marks were not retained as long as V.I. tags or adipose clips, and that the visibility of V.I. marks varied depending on light intensity and location of the mark on the fish.

Unfortunately, branding, laser techniques, V.I. tags, and panjet marks require further research to determine their effects on physiology and fish behavior. Therefore, PIT tags were chosen for mark/recapture studies described in this report, since these tags allow nonintrusive identification of treatment fish at recapture weirs.

Several pilot investigations were undertaken prior to commencement of full-scale NATURES research.

The NMFS Newport Laboratory conducted laboratory research on the feasibility of conditioning salmonids to avoid predators (Section 11). In these studies, coho salmon (*O. kisutch*) disturbed by physical stressors demonstrated higher blood cortisol levels and vulnerability to predation by lingcod (Ophiodon *elongatus*) than non-stressed fish. Spring chinook salmon that had prior exposure to predation were less vulnerable to predation when compared with those that had not been previously exposed. Antipredator conditioning and stress reduction appeared to be keys for ameliorating the negative impacts of hatchery rearing on postrelease survival for juvenile salmon.

Nevertheless, a subsequent experiment (Section 9) failed to demonstrate the efficacy of predator conditioning in improving postrelease instream survival of fall chinook salmon. For this study, fish were reared to age-0 srnolts using standard fish culture techniques. Test groups were then allocated to one of two identical 2.2-m diameter circular tanks; the "training" tank held two predatory cutthroat trout (0. *clarki*) whereas the control tank had no predators. The fish were held under these conditions for 16 hours prior to release into a small coastal stream. This procedure was replicated six times. There was no significant difference in the proportion of trained and untrained smolts recovered at a downstream weir.

It is possible that antipredator training procedures used in this study were not extensive enough to improve antipredator recognition or antipredation responses. Future NATURES research studies will focus on developing methods to successfully train salmon smolts to avoid the most significant predator(s) they are likely to encounter after release, which, depending on the postrelease environment, may include piscine, avian, or even terrestrial predators.

The USFWS Abernathy Salmon Culture Technology Center reviewed information regarding feeds and feed delivery systems designed to reduce stress in hatchery fish (Section 12). Factors controlling feeding behavior of wild salmon include vision, olfaction, taste, diel and seasonal feeding patterns, and prey characteristics. All of these factors must be addressed in developing a new generation of hatchery fish food, and this can be done by use of live feeds and/or developing artificial feeds with diverse shapes, textures, colors, and scents that elicit wild-like feeding responses in the fish.

Feed extrusion technology offers the ability to produce commercial feeds with wild food attributes. For instance, long, thin pellets can be produced, which have been shown to elicit stronger feeding responses than standard pellet shapes. Ideally, feed should be delivered below the surface in a drift form with enough current to keep it in suspension. Feed should also be delivered in low volumes, at high frequency, and at random subsurface locations throughout the raceway to simulate invertebrate drift patterns and to minimize territorial behavior and aggression in fish.

The NMFS Manchester Laboratory initially investigated the use of live-food supplementation to increase the postrelease foraging ability of hatchery-reared fall chinook salmon (Section 4). Replicate groups of fry were reared in six 2.4-m-diameter circular tanks and fed on

two different diets. Fish in three tanks received a standard, commercially available, pelletized diet, while those in the other tanks were given the opportunity to forage on natural live prey (mysids, mosquito larvae, chironomid larvae, and daphnia) prior to their daily ration of pellets. When foraging ability of individual fish was examined in 200-L observation tanks, the trained salmon were found to feed on twice the number of familiar prey (chironomids) and novel prey (mayfly larvae) as untrained fish. This work suggested that live-food supplementation could be used to increase the postrelease foraging ability of hatchery-reared salmon.

Nevertheless, a subsequent experiment (Section 8) failed to demonstrate the efficacy of live-food supplementation in improving instream foraging efficiency of spring chinook salmon. In this experiment, 24 replicate groups of yearling fish were held in 400-L tanks for approximately the last 60 days of rearing. The fish in all tanks received an equal volume of feed pellets each day. Fish in 12 tanks were given an additional ration of brine shrimp or tubifex worms prior to being fed pellets. At the end of the rearing period, the foraging efficiency of groups of test and control fish was evaluated in both freshwater and marine test arenas by allowing the fish to forage on natural prey for about 1 week

Comparison of stomach contents from fish in the experiments indicated no significant difference in trained and untrained fish. Given observations of successful forage training with other species, it is surprising that habitat enrichment in this study had no effect on postrelease foraging ability. However, this observation may have been the result of very few fish in the study feeding, since many fish had little digestible material in their stomachs and most did not appear to have been feeding as well as they should

For habitat enrichment to enhance foraging ability, it may be necessary to instill a preference for live food diets earlier in the rearing cycle of salmonids. Future NATURES research will determine if feeding live-food supplemented diets fed from swimup to release is a better approach for enhancing postrelease foraging success of hatchery-reared fish.

The NMFS Manchester Laboratory evaluated the effectiveness of various components of NATURES habitat concepts in three postrelease survival experiments conducted on chinook salmon (Sections 5-7). In the first experiment (Section 5), fall chinook salmon were reared for 4 months from swimup to smoltification. These fish, which were cultured in 400-L raceways outfitted with cover, structure, and substrate, survived instream travel to a collection weir 2.2 km downstream at a rate 50% higher than conventionally reared salmon. In the second experiment (Section 6), spring chinook salmon were reared for 3 months in 400-L raceways outfitted with cover, structure, and substrate. In clear water, these fish survived at a rate 24% higher than controls after release and traveling 225 m downstream to a collection weir. However, when fish were released in turbid water conditions, there was no significant difference in postrelease survival between test fish and controls.

In the final experiment (Section 7) conducted in conjunction with WDFW, culture vessel size was increased to 5,947 L, and fall chinook salmon were reared for about 4 months from swimup to smoltification. NATURES raceways were outfitted with similar types of cover, structure, and substrate used in the other two experiments. However, in this study, an underwater feed delivery system was added to the NATURES treatment. In this experiment, NATURES fish averaged 27% higher postrelease survival to a collection weir 21 km downstream than their conventionally reared counterparts.

In these studies (Sections 5-7), the NATURES variables tested succeeded in producing more "wild-like" fish than conventional rearing methods. NATURES fish developed light and

dark mottled body camouflage coloration patterns that were cryptic for the diverse stream bottom background over which these fish were released. In contrast, the uniformly light colored., conventionally reared fish were cryptically mismatched for their release environment and required over 1 week of stream residence to begin development of the long-term color adaptations that can provide cryptic camouflage coloration for the stream background. By our subjective observations, the NATURES fish also displayed a greater fright response to overhead movement than the conventionally reared groups.

The high prerelease survival (98%+) of both conventionally- and NATURES-reared fish in all studies suggests that the NATURES culture techniques we tested do not adversely affect fish health.

We believe the 25-50% survival advantage during migration in the stream corridor for most groups of NATURES fish was primarily due to the external camouflage color patterns of NATURES fish, which probably reduced their susceptibility to predation by visually hunting predators (e.g., birds and other fish). This may be why survival advantages were not noted for NATURES fish released in turbid water conditions (Section 6) where relatively visibility was reduced. However, in the last experiment, it is probable that the automated underwater feeding system also lessened predator vulnerability of NATURES fish by inducing benthic orientation.

Our research has demonstrated that rearing-habitat modification techniques developed in pilot-scale NATURES studies can be implemented in production fish-rearing. We have demonstrated that modification of the culture environment can produce significant positive differences in behavior and postrelease survival of hatchery fish in streams. The research conducted for this report demonstrates that rearing chinook salmon in NATURES environments with substrate, in-stream structure, and overhead cover increases instream postrelease sutvival. Our research also suggests thatproviding feed in the water column instead of at the surface can enhance fish foraging behavior. This is an important step in developing NATURES culture habitats for producing "wild-like" fish from hatcheries for use in genetic conservation and supplementation programs.

NATURES techniques have been designed to be retrofitted to existing hatchery raceway systems and vacuuming substrates is the only NATURES raceway operation procedure requiring significant increased maintenance effort. Given the demonstrated benefits of NATURES rearing on juvenile postrelease survival, without any demonstrated fish health costs, we believe NATURES should continue to be developed for production-scale use.

Future research should determine which experimental variable (e.g., substrate, instream structure, overhead cover, feed delivery, live-food supplementation, predator training, etc.) provides the greatest postrelease survival benefit. Studies are also needed to determine the interactive effect between all the experimental variables selected for inclusion in NATURES rearing. In addition, future research should focus on developing live-food supplementation diets that promote enhanced foraging ability for a broad spectrum of natural prey. Research is required to verify that live-food supplementation increases foraging ability, growth, and survival in postrelease environments.

Importantly, future research should determine whether the instream survival benefits demonstrated for NATURES smolts translates into increased recruitment to the fishery and for escapement. Because of the low adult returns of Pacific salmon in the Columbia River Basin, adult survival experiments must be conducted at production level, utilizing most of the resources of one or more hatcheries for several years.

The success of salmonid culture programs is now measured primarily by increasing the prerelease survival of salmonid fishes. NATURES complements this methodology by concentrating on husbandry aspects that can increase postrelease survival. We concluded that these innovative culture techniques are effective and have potential benefit for both enhancement and conservation hatcheries.

NATURES research provides a foundation for development of conservation hatchery concepts necessary for protection of native fish when hatchery operations conflict with wild populations of Pacific salmon. NATURES strategies should provide "wild-like" hatchery fish that are more suitable for use in supplementation programs than conventionally reared fish and should also help minimize potential genetic divergence between wild and hatchery-reared salmonids. NATURES techniques have potentially broad application to restoration of depleted stocks, including those proposed for listing under the U.S. Endangered Species Act.

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PREFACE

Support for this research came from electrical rate payers of the Pacific Northwest through the Bonneville Power Administration.

DISCLAIMER

Reference to trade names does not imply endorsement by the National Marine Fisheries **Service,** NOAA.

INTRODUCTION

A goal of many fisheries restoration projects is enhancement of wild populations through release of hatchery-propagated fish. Unfortunately, most past attempts to use hatchery-reared fish to rebuild naturally-spawning populations of Pacific salmon have yielded poor results (Moring 1986, Miller 1990, Cuenco et al. 1993). Although the protective nature of hatchery rearing increases egg-to-smolt survival, the postrelease survival and reproductive success of cultured salmonids is often considerably lower than that of wild-reared fish (Greene 1952, Miller 1952, Salo and Bayliff 1958, Reimers 1963, Chilcote et al. 1986, Nickelson et al. 1986).

Hatchery practices that induce domestication are considered prime factors in reducing fitness of hatchery fish for subsistence in natural ecosystems (Reisenbichler and McIntyre 1977, Nickelson et al. 1986, Hillman and Mullan 1989, Goodman 1990, Waples 1991, Hilbom 1992). Present hatchery practices are geared toward mass-production under unnatural conditions (e.g., fish are reared in the open, over uniform concrete substrate; provided no structures behind or under which to seek refuge from water current, predators, or dominant conspecifics; held at high, stress-producing densities; surface fed, and conditioned to approach large, moving objects at the surface). It is probable that physiological, behavioral, and morphological modifications resulting from this unnatural rearing environment are major factors in the poor postrelease survival of many standard hatchery-reared salmon. For instance, hatchery fish often do not develop the proper cryptic coloration for the stream environment into which they will be released, and as a result, incur increased predation (Donnelly and Whoriskey 1991).

Nevertheless, supplementation (i.e., the use of artificial propagation in an attempt to maintain or increase natural production; RASP 1991) is a management strategy with potentially broad application to restoration of depleted stocks, including those proposed for listing under the U.S. Endangered Species Act. Theoretically, the fastest way to increase population numbers for depleted stocks of Pacific salmon is through release of hatchery-propagated fish to increase natural production. The challenge is in developing protocols that increase postrelease survival of hatchery-reared salmonids, thereby fostering successful supplementation.

It may be possible to develop culture systems that lower rearing stress and produce fish that are more fit for release. Pilot studies conducted by the National Marine Fisheries Service (NMFS) Manchester Marine Experimental Station indicated that fish reared in vessels, where cover and feeding strategies mimic natural conditions, display strong fright responses similar to those of wild fish (T. Flagg, NMFS, unpub. data). In addition, studies at the NMFS Newport Laboratory indicate that predator avoidance may be enhanced through behavioral conditioning.

We believe the production of quality "wild-like" fish from hatcheries can be achieved through development of a Natural Rearing Enhancement System (NATURES) which reduces domestication. The NATURES concepts includes rearing fish in raceways equipped with cover, structure, and natural substrates that promote development of proper body camouflage coloration; feed-delivery systems that condition fish to orient to the bottom rather than the surface of the rearing vessel; training of fish to avoid predators; exercising fish to enhance their ability to escape from predators; supplementing diets with natural, live foods to improve foraging ability; and reducing rearing densities. Development of natural rearing systems that minimize behavioral changes in hatchery-reared fish is identified as a priority [4.4.d] in the proposed Recovery Plan for Snake River salmon (Schmitten et al. 1995).

This report provides direction for development and evaluation of innovative culture techniques to produce "wild-like'* fish in hatcheries. In this report, we detail investigations made in collaboration with the Washington State Department of Fish and Wildlife (WDFW) and the U.S. Fish and Wildlife Service (USFWS) Abernathy Salmon Culture Technology Center. This is a collection of independent reports on interrelated aspects of NATURES research, which includes the following elements:

1) General experimental protocol for design and evaluation of NATURES (Section 1).

2) Comparison of hatchery and wild salmon biology and review of culture techniques for increasing postrelease survival (Sections 2 and 3).

3) Results and general discussion of experiments on various aspects of NATURES research (Sections 4-9).

4) Review of fish marking and tagging procedures suitable for NATURES evaluations (Section 10).

5) Review and research results on predator vulnerability of Pacific salmon (Section 11).

6) Review of feeds and feed delivery systems suitable for NATURES (Section 12).

NATURES research provides a foundation for development of conservation hatchery practices to protect native fish and to prevent hatchery operations from conflicting with the health of wild populations of Pacific salmon. NATURES strategies are intended to provide "wild-like" fish from hatcheries that are more suitable for use in supplementation programs than conventionally reared fish. NATURES strategies also should help minimize potential genetic divergence between wild and hatchery-reared salmonids. NATURES techniques have potentially broad application to restoration of depleted stocks, including those proposed for listing under the U.S. Endangered Species Act.

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Section 1

A GENERAL EXPERIMENTAL PLAN FOR EVALUATING CULTURE TECHNIQUES FOR INCREASING SUPPLEMENTAL FISH QUALITY AND POSTRELEASE SURVIVAL

by

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Discussion of Experimental Plan for NATURES

The Natural Rearing Enhancement System (NATURES) described in this report is a collection of experimental approaches designed to produce hatchery-reared chinook sahnon (*Oncorhynchus tshawytscha*) that exhibit wild-like behavior, physiology, and morphology. These strategies have application to fish restoration projects such as the Bonneville Power Administration's Yakima Fisheries Program.

Through our literature reviews, preliminary experiments, and practical experience, each described in following sections of this report, we have concluded that "wild-like" fish can be produced by rearing chinook salmon in seminatural culture environments. In the proposed NATURES program salmonids will be 1) raised in seminatural raceways with overhead cover, in-water structure, and natural substrate; 2) fed with an automated, subsurface food-delivery system; 3) reared on live, natural feeds; 4) exercised in high velocity currents; and 5) briefly exposed to predators. We assume that NATURES rearing will 1) promote the development of natural cryptic coloration and antipredator behavior; 2) increase foraging efficiency; 3) improve fish health by alleviating chronic, habitat-induced stress; and 4) reduce potential genetic selection pressures induced by the conventional salmon culture environment. Although founded on the best available scientific information, NATURES is an experimental treatment with high levels of uncertainty and risk.

The following experimental plan is designed to evaluate NATURES techniques that can be used in supplementation and conservation programs. The purpose of this research is to generate fish-culture and release techniques that yield high-survival, "wild-like" hatchery fish for supplementation. This will contribute to the rebuilding of depleted wild salmon stocks throughout the Columbia River Basin.

Overhead cover, substrate, in-stream structure, subsurface feeders, natural live diets, exercise, forage training, and predator conditioning were selected as NATURES culture strategies because they offer great promise for increasing postrelease survival and producing "wild-like" chinook salmon. However, these techniques have not been scientifically evaluated on a production scale. Thus, prior to basin-wide implementation, the merits of proposed NATURES culture strategies should be evaluated to determine if they significantly increase fishery and spawner recruitment, as well as reduce the genetic divergence between parental and cultured stock.

Because of the low adult returns of Pacific salmon in the Columbia River Basin, the ultimate adult survival experiment must be conducted at production level, utilizing most of the resources of one or more hatcheries for several years. The first step towards a large-scale process is to conduct pilot-scale research in laboratory vessels to ensure that the best form of each NATURES technique is incorporated into the production level experiment. Subsequently, production-scale research should be initiated to confirm or refute the effects of the selected techniques on adult survival.

Phase I pilot-scale research should evaluate each of the proposed NATURES experimental variables (overhead cover, substrate, in-stream structure, subsurface feeders, natural live diets, exercise, forage training and predator conditioning) to determine if they produce juvenile salmonids with behavior, physiology, morphology, and postrelease survival similar to wild-reared fish. This Phase I research should focus on determining the form of each experimental variable that is most efficient and practical for producing the desired effect. For example, in evaluating natural substrates, trials should be conducted with sand, pea gravel, cobble, exposed aggregate, and stream mural. After determining the best form of each experimental variable, research should

focus on evaluations of the interaction between the final selected culture methods. Only those methods that continue to produce a "wild-like" hatchery-reared salmonid with significantly increased postrelease survival should be incorporated into the production-scale evaluation.

In these evaluations, the experimental designs should include at least three treatments: 1) conventionally reared controls, 2) one or more seminaturally reared experimental fish, and 3) naturally-reared controls. In each experiment, all treatment groups must come from the same stock and must be randomly distributed to treatments at the initiation of experimental rearing. Wild stocks should be used whenever possible to comply with supplementation and conservation programs targeted for these stocks.

Use of substrate, overhead cover, instream structure, subsurface feeders, natural diets, exercise, forage training, and predator avoidance conditioning are the general experimental variables incorporated into NATURES research designs. However, as illustrated in Table 1- 1, a diverse array of alternative forms exists for each experimental variable. Control treatments for each experiment ate based on current standard practices at Columbia River Basin hatcheries. Once an experimental method is found to have the desired effect, research should be initiated to determine the ideal life-history stage and the exact duration that treatment(s) must be administered. Further pilot-scale research will determine which methods produce the desired effect in the most cost-effective form.

The biology of fish reared in conventional, NATURES, and natural environments will be compared to determine the ability of the NATURES rearing treatment to produce wild-like fish. Fish from each treatment will be assayed for behavioral (e.g., foraging, antipredator, social habitat, and migratory), physiological (e.g., stress, smolt, immunocompetence), morphological (e.g., coloration and morphometrics), and survival responses (Table l-2). Each of these response variables reflects an aspect of salmon biology that NATURES methods are expected to affect. By comparing fish from standard hatchery, NATURES, and wild rearing environments, investigators can determine if NATURES methods are successfully producing salmon with wild-like characteristics and can identify the relative importance of each method creating the overall NATURES approach.

Phase I studies should be conducted in laboratory raceways, with a sufficient number of fish to assess postrelease survival. At ponding, fish should be randomly distributed to the treatments, and randomization can be checked by comparing the mean fork length of fish in each treatment group at setup.

Treatments should consist of a conventional control, a naturally-reared control, and treatments for each form of NATURES experimental variable under consideration (Table l-l). For example, an experiment evaluating the effects of various substrate types would involve a group reared over conventional concrete (control); naturally-reared group (control); and NATURES groups reared over sand, pea gravel, exposed aggregate, and stream-bottom mural substrates. At least three (and preferably more) replicates are required per treatment, and a large number of rearing vessels (more than 15) ate required. Therefore, assuming scale is not a factor, smaller vessels (e.g., 400 L aquaria) may be advantageous, allowing for greater replication and treatments. However if scale is expected to effect a response, then larger vessels (such as pilot-scale raceways) will be required.

Table l-l. Potential forms of each generic experimental variable incorporated into the proposed Natural Rearing Enhancement System.

Substrate:

Concrete (conventional control)
Sand
Pea gravel
Cobble
Exposed aggregate
Stream bottom mural

In-stream Structure:

None (conventional control)
Artificial plants
Live aquatic plants
Cut conifers
Stick bundles
Pipes
Boulders
Stumps
Blocks

Overhead Cover:

Bird netting only (conventional control)
Solid (fabric, plastic, etc.)
Camouflage netting
Overhanging or floating plants

Feeders:

Hand surface (conventional control)
Automated surface (conventional control)
Automated midwater
Automated benthic

Diets:

Commercial pellets (conventional control)
Pelletized natural
Live natural
Commercial pellets with processed natural supplement
Commercial pellets with live feed supplement

Table l-l. Continued.

Exercise:

None (conventional control)
For full rearing duration:
  Intermittent at 1 body length/second
  Continuous at 1 body length/second
For partial rearing duration:
  Intermittent at 1 body length/second
  Continuous at 1 body length/second

Foraging training:

None (conventional control)
Live food only:
  full rearing duration
  partial rearing duration
Commercial pellet with live food supplement:
  full rearing duration
  partial rearing duration

Predator avoidance conditioning:

None (conventional control)
Live predator exposure with contact:
  Avian predators
  Fish predators
Live predator exposure without contact:
  Avian predators
  Fish predators
Stun model (electric shock) exposure
  Avian predator
  Fish predator
Predacious carnage video exposure
  Avian predator
  Fish predator

Table l-2. Response variables that must be measured to determine the effect of each NATURES experimental variable.

Response variable	Related experimental variables[a]							
	Habitat			Nutrition		Behavior/conditioning		
	Substrate	Structure	Cover	Feeders	Diets	Forage training	Exercise	Antipredator conditioning
Behavior:								
Social:								
Nips	Crit	Crit	Crit	Crit	Crit	Crit	NC	NC
Displays	Crit	Crit	Crit	Crit	Crit	Crit	NC	NC
Inter-fish distance	Crit	Crit	Crit	Crit	Crit	Crit	na	Crit
Polarization	Crit	Crit	Crit	Crit	Crit	Crit	na	NC
Inter-treatment dominance	Crit	Crit	Crit	Crit	Crit	Crit	Crit	NC
Foraging:								
Prey species	Crit	Crit	Crit	Crit	Crit	Crit	NC	Crit
Prey number	Crit	Crit	Crit	Crit	Crit	Crit	NC	Crit
Prey attack/stalk	Crit	Crit	na	na	Crit	Crit	NC	NC
Prey capture/attack	Crit	Crit	na	na	na	Crit	NC	NC
Prey ingestion/attack	na	na	na	na	na	Crit	NC	NC
Prey search time	Crit	Crit	na	Crit	na	Crit	NC	na
Prey handling time	Crit	Crit	na	na	Crit	Crit	NC	na
Stomach content weight	Crit	Crit	Crit	Crit	Crit	Crit	NC	Crit
Percent digestible material/stomach	Crit	na	na	na	Crit	Crit	NC	Crit
Percent nondigestible material/stomach	Crit	na	na	na	Crit	Crit	NC	NC
Anti-predator:								
Predator recognition distance	na	na	na	na	na	na	na	Crit
Response to cover	na	Crit	Crit	na	na	na	na	Crit
Predator evasion ability	Crit	na	Crit	na	NC	na	Crit	Crit
Burst Swimming ability	na	na	na	na	Crit	na	Crit	Crit

Table l-2 (continued).

Response variable	Related experimental variables[a]							
	Habitat			Nutrition		Behavior/conditioning		
	Substrate	Structure	Cover	Feeders	Diets	Forage training	Exercise	Antipredator conditioning
Behavior (continued):								
Number attacked by predator	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Number killed by predator	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Habitat preference:								
Distance from nearest structure	NC	Crit	NC	NC	NC	Crit	Crit	Crit
Distance from nearest streamside	NC	Crit	NC	NC	NC	Crit	Crit	Crit
Distance from bottom	NC	NC	Crit	Crit	NC	Crit	Crit	Crit
Migration:								
Travel time	NC	NC	NC	NC	NC	NC	Crit	Nc
Migration onset time	na	na	na	na	NC	NC	Crit	na
Cruise swimming speed	na	na	na	na	na	na	Crit	na
Morphology:								
Skin Coloration:								
Dorsal base hue	Crit	Crit	Crit	NC	Crit	NC	na	na
Dorsal base intensity	Crit	Crit	Crit	NC	Crit	NC	na	na
Dorsal base chromaticity	Crit	Crit	Crit	NC	Crit	NC	na	na
Parr mark darkness	Crit	Crit	Crit	NC	Crit	NC	na	na
Parr mark area/total body area	Crit	Crit	Crit	NC	Crit	NC	na	na
Number melanophores on anal fin	Crit	Crit	Crit	NC	Crit	NC	na	na
Number of dorsal spots	Crit	na	na	na	na	na	na	na

Table 1-2 (continued).

Response variable	Related experimental variables[a]							
	Habitat			Nutrition		Behavior/conditioning		
	Substrate	Structure	Cover	Feeders	Diets	Forage training	Exercise	Antipredator conditioning
Morphology (continued):								
Ventral iridescence	Crit	Crit	Crit	NC	Crit	NC	na	na
Morphometrics:								
Truss measurements	na	Crit	na	NC	Crit	NC	Crit	na
Fin condition	Crit	Crit	Crit	NC	Crit	NC	NC	na
Size								
Fork length	Crit	Crit	Crit	Crit	Crit	Crit	na	na
Standard length	Crit	Crit	Crit	Crit	Crit	Crit	na	na
Weight	Crit	Crit	Crit	Crit	Crit	Crit	na	na
Physiology:								
Gill Na-K ATPase	Crit	Crit	Crit	Crit	Crit	NC	Crit	NC
Thyroxine	Crit	Crit	Crit	Crit	Crit	NC	Crit	NC
Cortisol	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Liver glycogen	Crit	Crit	Crit	Cl-it	Crit	Crit	Crit	Crit
Immunocompetence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Hematocrit	Crit	Crit	Ctit	Crit	Crit	Crit	Crit	Crit
White cell count	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
% fat	na	na	na	NC	Crit	NC	na	na
% protein	na	na	na	NC	Crit	NC	na	na
% carbohydrate	na	na	na	NC	Crit	NC	na	na
% dry matter	na	na	na	NC	Crit	NC	na	na

Table l-2 (continued).

Response variable	Related experimental variables[a]							
	Habitat			Nutrition		Behavior/conditioning		
	Substrate	Structure	Cover	Feeders	Diets	Forage training	Exercise	Antipredator conditioning
Physiology (continued):								
% ash	na	na	na	NC	Crit	NC	na	na
Essential vitamins	na	na	na	NC	Crit	NC	na	na
Disease:								
BKD prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
IHN prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Columnaris prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Bacterial Gill prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Saprolegnia prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Furunculosis prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Red Mouth prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Cold water prevalence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Reproduction:								
Maturation:								
Gonadosomatic index	NC	NC	NC	NC	Crit	Crit	Crit	na
Primary sexual character:								
Egg diameter	NC	NC	NC	NC	Crit	Crit	Crit	na
Sperm motility	NC	NC	NC	NC	Crit	Crit	na	na
Fecundity	NC	NC	NC	NC	Crit	na	na	na

Table l-2 (continued).

Response variable	Related experimental variables[a]							
	Habitat			Nutrition		Behavior/conditioning		
	Substrate	Structure	Cover	Feeders	Diets	Forage training	Exercise	Antipredator conditioning
Reproduction (continued):								
Secondary sexual characteristics:								
Nuptial tooth/standard length	NC	NC	NC	NC	Crit	NC	na	na
Nuptial coloration	NC	NC	NC	NC	Crit	Crit	na	na
Kype size	na	na	na	na	Crit	Crit	Crit	na
Spawning success:								
Number of fertilized eggs	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Number of swimup fry	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Reproductive behavior:								
Female redd construction	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Female redd defence	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Male spawning success	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Age at maturation	na	na	na	Crit	Crit	Crit	Crit	na
Number of spawning adults	na	na	na	Crit	Crit	Crit	Crit	na
Survival:								
In culture (NATURES and control)	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
In stream (Wild)	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Postrelease smolt	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Fishery recruit	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Adult return	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit
Adult spawn	Crit	Crit	Crit	Crit	Crit	Crit	Crit	Crit

[a] Crit = critical for testing, NC = secondary in importance for testing, na = not applicable.

Phase I study fish should be reared in these treatments from swimup until smolting. During this rearing period, the appropriate response variables should be measured to evaluate treatment effects. For example, for a substrate evaluation, the social behavior, foraging behavior, skin coloration, size, disease, and in-culture survival should be measured for fish in each treatment to determine which experimental form produces the most wild-like fish. The amount of fouling and cleaning effort for each treatment should also be compared.

Just prior to release, subsamples from each treatment should be assessed for all response variables of interest. A representative subsample ($n > 30$) of fish should be sacrificed and assayed for skin coloration, morphometrics, fin condition, and disease status. Another representative subsample ($n > 30$) should be evaluated for foraging, habitat preference, and antipredation behavior to provide basic information to assess how each rearing treatment affected the fish.

Finally, the remaining fish that have been reared under various treatments should be tagged and released into a natural migratory corridor, where their migratory behavior and postrelease survival can be evaluated. Postrelease survival information is the most important factor in evaluating effectiveness. However, the assumption that recovery represents postrelease juvenile survival can only be validated when representative samples of migratory and residualizcd fish are collected. Weir blow-outs during freshets, instream sampling equipment biases, and river size usually erode our confidence in this assumption. However, confidence in this assumption can be maximized by selecting stream and riverine systems with the highest and least-biased recapture rates of migratory and residualized experimental fish..

Once full-term rearing with NATURES has produced improved survival, Phase I research should then switch to determining whether administration of the treatment for shorter periods produces similar effects. Exposure time and life-history stages of treatment initiation should be evaluated with experimental treatments that include 1) full-term exposure, 2) limited exposure initiated just after swimup, 3) limited exposure initiated just prior to release, and 4) limited exposure initiated midway through rearing.

Phase II production-scale research should be conducted at a site that has facilities for at least two fish-culture treatments (e.g., conventional and NATURES) and that is located near the natural rearing area for the experimental wild stock. A wild stock should be used to maximize the probability that results can be applied to fish-culture programs designed to restore and build self-sustaining natural runs. The wild stock must be randomly distributed to all experimental rearing treatments to verify that any differences observed are due to treatment and not variation due to sampling bias.

The experimental culture facility (e.g., raceway dimensions) should be similar to most other Columbia River Basin salmon production facilities. This similarity will facilitate technology transfer by allowing experimental concepts with proven value to be easily retrofitted to other facilities. In order to ensure that the treatments vessels do not introduce systematic bias to the evaluations, all rearing vessels must be identical and must accommodate both the control and experimental methods of all treatments.

There should be sufficient replicates per treatment to provide a reasonable chance of statistically significant differences between treatments, both in recruitment to the fishery and in adult returns to the hatchery rack An alpha level of 0.05 is the ideal for these determinations, although an alpha level of 0.10 might be considered if it is the only way to obtain an answer in no more than one rearing cycle (4-5 years). With appropriate experimental design, replicates may be spread over several years or facilities to provide a quicker turnaround time or reduce demands on

any one facility. If replicates are to be released at different times or places, then an appropriate block design can be applied (replicates grouped by release site, release time, release year, etc.) for statistical analysis.

Section 2

A COMPARISON OF HATCHERY AND WILD SALMONID BIOLOGY

by

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Contents

Introduction

This review examines the existence and potential causes of behavioral, morphological, survival, and reproductive differences between wild and hatchery salmonids. Comparing the behavioral ecology of wild and hatchery fish provides insight into the mechanisms controlling their survival and reproduction. Identifying the genetic and environmentally induced components of survival-related attributes provides useful information for developing fish culture practices that minimize differences between wild and hatchery fish.

Survival

Studies during the 1950s and early 1960s were the first to document that the survival of hatchery-reared fish released in the natural environment is often significantly lower than that of their wild-reared counterparts. Over several years, Greene (1952) recovered wild brook trout fingerlings (*Salvelinus fontinalis*) at rates 8.4 to 18.6 times higher than those of hatchery fingerlings planted in the same lake. Reimers (1963) found that 30% of rainbow trout (*Oncorhynchus mykiss*) planted in streams died within 44 days of release, while only one wild trout died during the same period. Salo and Bayliff (1958) found the survival of seaward migrating wild coho salmon (0. kisutch) smolts was three times better than that of their hatchery-reared counterparts. In these studies, it is unclear whether survival differences between hatchery- and wild-reared fish were primarily the result of genetic or environmental differences, or whether differences were due to some combination of the two factors.

Miller (1953) found that only 5% of the hatchery-reared cutthroat trout (0. *clarki*) he planted in streams survived a year after release, while 46% of the transplanted wild trout survived during the same period. In the same study, stream-reared hatchery fish had an intermediate survival value of 17.2% (Miller 1953). This suggests both genetic strain and rearing environment play a role in the postrelease survival of hatchery produced fish.

Research conducted since the mid-1960s also suggests that the poor postrelease survival of hatchery fish represents both adaptive differences between hatchery and wild populations and environmental differences between hatchery and natural rearing environments. When Mason et al. (1967) compared survival of wild, domestic, and hybrid strains of brook trout, reared from fertilization to parr under identical environmental conditions, they found that each pure strain was best adapted to its own rearing environment. In this study, wild brook trout showed the poorest growth and survival in the hatchery, but 10.2% survived when released into test stream sections compared to 3.6% survival for hybrid trout and a 0.7% rate for domestic trout. Apparently, the rearing environment also affected postrelease survival in this study, since nearly twice as many naturally reared wild fish survived as hatchery-reared wild fish (19.7 vs. 10.2%) after both were transplanted into test stream sections.

Fraser (198 1) conducted a study in which he stocked equivalent numbers of domestic, wild, and domestic/wild hybrid strains of brook trout in nine precambrian lakes. In six of the nine lakes, he recovered wild fish at two to four times the rate of domestic fish. In the remaining three lakes, the recovery rate was similar for all three strains. In a subsequent study, Fraser (1989) concluded that wild and hybrid strains were able to establish self- perpetuating breeding populations more frequently than pure domestic strains released into the same lakes.

LaChance and Magnan (1990a, b) found that wild and hybrid strains of brook trout survived in lucustarine habitats better than a domestic strain planted in the lake during the same

period. All three strains were reared from eggs in the hatchery before being released into lakes and were influenced by the presence of intra- and interspecific competitors.

Poor survival of both hatchery strains in natural environments and wild strains in hatchery environments suggests that in many cases selection has resulted in the genetic divergence of hatchery populations from their wild ancestors. Reisenbichler and McIntyre (1977) examined the growth and survival of hatchery, wild, and hatchery-wild hybrid strains of steelhead trout reared from eggs under identical conditions in streams and a hatchery pond. Again, each pure strain was best adapted to its environment, with more wild fish surviving in the stream and more hatchery fish surviving in the hatchery. Hybrid fish surviving in the stream grew faster than their wild counterparts, whereas hatchery fish grew faster in the hatchery pond

In other studies, the naturally spawned and reared offspring of hatchery steelhead experienced greater mortality than the offspring of wild steelhead during all major (egg-to-fry, fry-to-smolt, and smolt-to-adult) life history stages (Chilcote et al. 1986, Leider et al. 1990). These studies strongly suggest that adaptive differences occurred between hatchery and wild populations in a relatively short evolutionary time period.

Foraging Behavior

Starvation is a primary cause of poor postrelease survival in hatchery fish. Miller (1952) believed the high mortality of hatchery cutthroat trout in his studies was due to starvation. Hochachka (1961) reached a similar conclusion after examining the stomach contents of wild and hatchery trout 28 days after release into a stream: he found the latter group had less food in their stomachs and lower mean body weights. Reimers (1963) concluded that the continuous and eventually lethal weight loss he observed in hatchery trout resulted from their inability to compete or forage in the wild.

Sosiak et al. (1979) concluded that hatchery-reared Atlantic salmon (*Salmo salar*) parr foraged less effectively than naturally-reared parr for at least 2 months after they were released into streams. The wild parr consumed more food and a greater diversity of organisms than their hatchery counterparts. In addition, the wild parr fed primarily on benthic organisms while the hatchery-reared fish concentrated on terrestrial and winged insects: this suggested that hatchery parr continue to feed at the surface even after release. O'Grady (1983) also found that hatchery-reared trout ate less than their naturally-reared counterparts immediately after release.

Meyers (1980) found that hatchery chinook salmon (0. *tshawytscha*), examined shortly after their release, were inept foragers compared to wild fish. The average ratio of stomach content to body weight was more than three times greater in wild fish (5.7%) than in hatchery fish (1.7%). In addition, these newly released hatchery fish appeared to be nonselective feeders, with 67% of their stomach contents being indigestible algae compared to 84% anchovy (*Engralus mordax*) in the stomachs of wild chinook salmon. After extended residence in the estuary, the diet composition of hatchery and wild chinook salmon converged, suggesting that hatchery fish eventually learned to forage more efficiently. However, this change may also have resulted from starvation of inept foragers, leaving only more efficient foragers to be sampled.

At least two studies suggest that foraging differences between wild and hatchery strains of salmonids are partially innate. Mason et al. (1967) found that wild, hatchery, and hybrid strains of brook trout, reared from eggs in identical environments, exhibited different foraging behaviors: wild-strain fish fed only from the bottom, while hatchery fish readily fed at the surface, and

hybrids exhibited intermediate behavior. Uchida et al. (1989) also found innate differences in the foraging behavior of wild and domestic strains of ayu (*Plecoglossus altivelis*) larvae reared under identical conditions. Again, the hatchery strain readily fed from the surface while the wild strains would not.

Other studies suggest foraging differences between wild and. hatchery-reared fish are affected by conditioning. Hatchery-reared brown trout (*S. trutta*) that were released into a lake fed initially on surface-dwelling prey (Johnson and Ugedall986). However, after several weeks of lake residency the fish apparently learned to feed on natural prey, and in later analyses the diets of both wild and hatchery-reared trout were similar. In this study, the percentage of inedible and energetically unprofitable items eaten by hatchery-reared trout decreased over time, suggesting that foraging efficiency can be improved with experience (Johnson and Ugedal 1986).

Regardless of rearing environment or strain, the salmonids studied by Bryan (1973), as well as those studied by Paszkowski and Olla (1985), always preferred live prey over commercial pellet diets. This suggests that prey movement is a primary cue stimulating prey attack behavior. While Bryan (1973) found rainbow trout had an innate preference for live prey over pellets, he also determined that fish developed weak and readily reversible training biases for familiar foods over novel foods. He concluded that cues other than familiarity were probably important in the natural foraging behavior of trout.

Paszkowski and Olla (1985) demonstrated that experience with live prey improved the foraging performance of hatchery-reared coho salmon smolts challenged to feed on Crugon spp. However, some smolts, even after repeated strikes, were never able to ingest large Crugon, though they were within ingestible size limits for the fish. Overall, these studies indicate that live food supplementation maybe useful in training salmon to handle live prey more efficiently and in preventing the development of dietary preference against natural feeds.

Habitat Preference

Cultured and naturally-reared salmonids also respond differently to habitat. Allee (1974) found that wild coho salmon utilized both riffles and pools, while hatchery-reared coho sahnon primarily used pools. In an artificial stream channel, hatchery-reared Atlantic salmon parr persistently held positions higher in the water column than naturally reared parr from the same parent population, indicating that the hatchery rearing environment caused a shift in habitat preference (Dickson and MacCrimmon 1982). Hatchery brown trout released into a study stream used less energetically efficient foraging sites than wild trout, even though they frequently displaced wild trout from these sites (Bachman 1984). The hatchery trout also had higher energy costs as they constantly moved from site to site.

Typically, hatchery strains are more surface-oriented than wild strains. The cultured Atlantic salmon parr observed by Sosiak (1978) swam closer to the surface and spent more time in contact with the surface than wild parr. Similarly, Mason et al. (1967) found hatchery strains of trout were more surface-oriented than wild strains reared from eggs in the same environment. Uchida et al. (1989) observed that wild ayu larvae were found at greater depths than hatchery larvae reared and observed in the same environment. Most of the innate surface orientation of hatchery fish is probably an adaptive response to the common culture practice of introducing food at the surface.

Social Behavior

Juvenile salmonids establish and defend foraging territories through agonistic contests, and levels of aggression have been positively associated with dominance in these contests (Egglishaw 1967, Fenderson and Carpenter 1971, Holtby et al. 1993, Berejikian 1995a,b). Dominant individuals tend to obtain more energetically profitable stream positions (Fausch 1984, Metcalfe 1986); hence, fish with relatively high levels of aggression may be expected to have a competitive advantage over less aggressive fish.

Evidence suggests that agonistic behavior has a genetic basis, but can be profoundly influenced by environmental (rearing) conditions. In a comparison between hatchery and naturally reared Atlantic salmon of common ancestry, agonistic activity of hatchery-reared fry was greater than that of naturally reared fry over a range of rearing densities, while wild fry were more aggressive at only the lowest densities (Fenderson et al. 1968, Fenderson and Carpenter 1971). Hatchery-reared brown trout were equally successful in agonistic contests against wild brown trout in a natural stream, but hatchery trout abandoned their territories and moved more frequently among territories than did wild trout (Bachman 1984).

These studies indicate that hatchery rearing environments can profoundly influence social behavior. Food availability and rearing densities in hatcheries typically far exceed those in natural streams, which may partly account for differences in agonistic behavior between hatchery and naturally reared fish. Internal motivational state (e.g., hunger) is positively associated with aggression (Symons 1968, Olla et al. 1990), and territorial hierarchies can break down at high social densities (Grant and Kramer 1990).

Levels of aggression appear to differ between domesticated and wild populations, suggesting that genetically based changes can occur in a hatchery population after only a few generations of culture. Offspring from a domesticated brook trout population demonstrated higher levels of aggressive activity than offspring from a wild population when both populations were reared under similar hatchery conditions (Moyle 1969). Newly emerged, "socially-naive" coho salmon fry from two domesticated populations demonstrated significantly greater levels of aggression than fry from geographically proximate wild populations (Swain and Riddell 1990). In a companion study, aggression in coho salmon was found to be a heritable trait (Riddell and Swain 1991). The results of these studies demonstrate a genetic basis for the differences found between hatchery and wild populations.

Berejikian (1995a,b) suggested that newly emerged fry from a wild steclhead population initially had higher levels of aggression than fry from a locally derived, domesticated population. However, after several months of rearing, offspring of domestic steelhead were significantly more aggressive than offspring of wild steelhead when both were teared in food-limited and/or low-density environments (including a natural stream channel).

Thus, juvenile salmonids from domesticated and wild populations appear to demonstrate adaptive differences in agonistic behavior, and the behavioral development of domesticated and wild fish appears dependent upon their rearing environment.

Reproductive Behavior

Hatchery practices have altered reproductive behavior by relaxing selection pressure on secondary sexual characteristics that are used in breeding competition in the wild, while increasing selection pressure on primary sexual characteristics. Fleming and Gross (1989) concluded that relaxation of breeding competition in the hatchery has led to the evolution of female coho salmon with less well developed kypes and breeding colors than their wild counterparts. The hatchery strains they studied expended their energy in developing larger and more numerous eggs than equivalent size members of the wild stocks from which they were derived

The reproductive behavior of male coho salmon also differs between hatchery and wild strains (Fleming and Gross 1992). Hatchery-strain males that were allowed to spawn naturally were less aggressive and were generally less active than wild-strain males. It appeared that the relaxation of competition among males for access to females in the hatchery, coupled with the possibility of sperm competition that may have occurred as a result of hatchery spawning techniques, resulted in hatchery-strain males investing disproportionate amounts of energy towards testes production. The authors concluded that investing energy for sperm production rather than in secondary sexual characteristics that aid in obtaining access to females was only a disadvantage to hatchery-strain males spawning naturally in the presence of wild-strain males. In the absence of competition, hatchery-strain males would probably breed as successfully as wild-strain males.

Either inadvertently or intentionally, hatcheries usually develop strains which spawn at different times than their ancestral stocks. Studies by Salo and Bayliff (1958), Ricker (1972), and Hager and Hopley (1981) have all demonstrated a genetic basis for spawning time. Hatcheries often inadvertently select for early run timing by spawning a disproportionately higher percentage of earlier returning fish. From a management perspective, the advantage of this temporal separation is that it minimizes interbreeding between domestic and wild stocks, which is generally believed to be harmful to wild populations (Reisenbichler in press). The disadvantage is that the progeny of feral-spawning domestic strains emerge prior to peak abundance of natural aquatic invertebrate blooms, and thus suffer high mortality rates (Nickelson et al. 1986).

Response to Predators

Predation is a major factor affecting the postrelease survival of hatchery-reared fish. Ellis and Noble (1960) estimated 12-30% of the juvenile chinook salmon released from the Washington State Department of Fisheries hatchery on the Klickitat River were preyed on in the 40 miles between the hatchery and the Columbia River. In the Chehalis River in western Washington State, hatchery coho salmon were more vulnerable to squawfish (*Ptychochelius oregonensis*) predation than wild coho salmon, with squawfish rarely feeding on smolts until they were released from the hatchery (William Waknitz, NMFS, pers. commun., July 1991).

Other evidence also indicates that hatchery strains may be more vulnerable to predation than wild strains. Offspring of crosses between wild steelhead and hatchery-strain rainbow trout were more willing to forage in the presence of a predator (an adult rainbow trout) than offspring of pure wild steelhead crosses. However, hybrids were no better able to avoid predation in 15-second trials (Johnsson and Abrahams 1991).

Increased risk-taking behavior without an increased ability to avoid predators may have placed domesticated rainbow trout at greater risk of predation than wild steelhead Wild steelhead fry from the Quinault River in Washington State were better able to avoid predation by prickly

sculpin (*Cottus asper*) in three separate experiments than fry from a locally derived hatchery population (Berejikian 1995a,b). In both studies, fry were reared under laboratory conditions, so behavioral differences between hatchery and wild populations were probably genetically based.

Fish that approach the surface of the water column are known to have a greater risk of avian predation (Kramer et al. 1983). Therefore, the surface orientation of cultured fish, and their tendency to approach large moving objects at the surface, may increase the probability of their being preyed on by herons (*Ardea herodias*), mergansers (*Lophodytes cucullatus*, *Mergus* merganser, and M. *serrator*), and other avian predators. Mason et al. (1967) reared both hatchery and wild strains of brook trout in raceways and observed that wild-strain trout fled caretakers, while hatchery strain trout approached them: this suggested that the tendency of hatchery fish to approach large moving objects is partly innate.

Other studies have shown that fright responses are at least partially a conditioned behavior (Patten 1977, Olla and Davis 1989). Cultured cod (*Gadus morhua*) approached larger cod more slowly and less closely than wild cod (Nordeide and Svassand 1990). The investigators speculated that these cultured fish experienced greater cannibalism in their rearing environment and thus became conditioned to avoid potentially cannibalistic larger cod.

In the laboratory, Barns (1967) observed that naturally reared sockeye salmon (*O. nerka*) fry were less susceptible to predators than hatchery-reared fry. This susceptibility was inversely related to the proportion of time alevins were reared with gravel in their incubation baskets. In these studies, the vulnerability to predation was size-related, and since the fry reared in baskets without gravel were smaller, Bams (1967) concluded that the rearing environment was responsible for increased predation on hatchery fry.

Morphological and Physiological Differences

Taylor and Larkin (1986) developed a discriminate function model using morphometric measurements to distinguish between hatchery and naturally reared coho salmon parr. In addition to having a different shape, hatchery reared fish were less variable than naturally reared parr. Taylor and Larkin (1986) concluded that these differences were under environmental rather than genetic control. Bams (1967) and Taylor and McPhail(l985) indicated that hatchery-induced morphological differences can affect swimming speed and ability to escape predators.

Jarvis (1990) determined that predator-naive Atlantic salmon smolts facing a new osmotic environment suffered more severe physiological stress when predators were present than did smolts previously exposed to predators. These and other morphological and physiological divergences between natural and hatchery-produced fish may significantly influence postrelease survival

Conclusions

Artificial culture environments condition salmonids to respond to food, habitat, conspecifics, and predators in a different manner than do fish reared in natural environments. Present culture techniques also alter selection regimes, which result in genetic divergence between hatchery and wild populations. Therefore, to ensure maximum success, supplementation programs should use locally adapted, wild broodstocks. Offspring of wild broodstocks should be cultured in rearing environments that promote the natural development of important survival-related

behaviors and that minimize domestication selection. The success of supplementation programs hinges on both utilizing wild broodstocks and developing these new rearing techniques.

The phenotypic differences observed between cultured and wild fish are both genetically and environmentally controlled, and these differences may be reduced by altering the hatchery rearing environment to produce a more "wild-like" fish. Hatchery rearing techniques that might minimize genetically based and environmentally induced behavioral changes are discussed in the following sections of this report.

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Section 3

A REVIEW OF SEMINATURAL CULTURE STRATEGIES FOR ENHANCING THE POSTRELEASE SURVIVAL OF ANADROMOUS SALMONIDS

by

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Contents

Introduction

The success of sahnonid culture programs is now achieved primarily by increasing the prerelease survival of salmonid fishes. Artificial propagation may increase egg-to-smolt survival by more than an order of magnitude over that experienced by wild fish. Unfortunately, the postrelease survival of these cultured salmonids is often considerably lower than that of wild-reared fish (Greene 1952, Miller 1952, Salo and Bayliff 1958, Reimers 1963). Whereas this low postrelease survival may be acceptable in put-and-take fisheries, it is intolerable in supplementation programs designed to rebuild self-sustaining natural runs and conserve genetic resources. Continued success of hatchery programs can be assured by implementing innovative fish culture techniques that increase the postrelease survival of hatchery salmonids.

Releases of hatchery strains of brook trout (*Salvelinus fontinalis*) failed to recolonize vacant habitats; however, releases of wild strains usually succeeded (LaChance and Magnan 1990a). Similarly, the use of hatchery coho salmon (Oncorhynchus *kisutch*) to supplement natural runs can cause a long-term decline in stream production (Nickelson et al. 1986). Low postrelease survival of hatchery salmonids compared to their wild cohorts may result from the behavioral and morphological differences that develop in cultured fish. For example, the practice of feeding pellets at the surface by hand or from vehicles results in hatchery brook trout and Atlantic salmon (*Salmo salar*) that are more surface oriented and more likely to approach large moving objects than wild fish (Mason et al. 1967, Sosiak 1978).

This surface orientation makes these hatchery-reared sahnonids more vulnerable to avian predators (e.g., herons, kingfishers, and mergansers). The conventional hatchery environment also produces brook trout, brown trout (S. *trutta*), and coho salmon with more aggressive social behavior than is evident in wild-reared fish (Fenderson et al. 1968, Bachman 1984, Swain and Riddell 1990). After release, the heightened aggressive tendencies of these hatchery fish put them at a greater risk from predation and often result in inefficient expenditure of energy in contests over quickly abandoned feeding territories. In addition, many hatchery salmonids exhibit inept foraging behavior that results in their stomachs containing fewer digestible items than those of their wild-reared counterparts (Miller 1953, Hochachka 1961, Reimers 1963, Sosiak et al. 1979, Myers 1980, O'Grady 1983).

As adults, hatchery strains of coho salmon have better developed primary sexual characteristics (egg size and number), but less well-developed secondary sexual characteristics (kype size and nuptial coloration) than do wild-reared strains (Fleming and Gross 1989). These reduced secondary sexual characteristics of hatchery strains may prohibit their ability. to defend redd sites when spawning naturally. Although the effect on postrelease survival is unknown, the shape of hatchery and wild chinook salmon (0. *tshawytscha*) also differs at the juvenile stage (Taylor 1986).

Phenotypic differences observed between cultured and wild fish are both genetically and environmentally induced. The artificial culture environment conditions salmon to respond to food, habitat, conspecifics, and objects in a different manner than would the natural environment. Present culture techniques also alter selection pressures, which results in cultured strains becoming innately distinct from wild strains (Flick and Webster 1964, Fraser 1981, 1989; LaChance and Magnan 1990b; Mason et al. 1967; Reisenbichler and McIntyre 1977; Swain and Riddell 1990).

Theoretically, both environmental conditioning and shifts in evolutionary selection pressure produced by the artificial culture environment can be alleviated with culture practices that simulate a more natural rearing environment. In this section, we review fish culture methods for increasing

postrelease survival. The use of antipredator conditioning, foraging training, supplemental dissolved oxygen, and reduced rearing density will be examined.

Antipredator Conditioning

Predation may be a key factor in the poor postrelease survival of cultured salmonids. The ability of an animal to avoid predation is dependent on proper cryptic coloration to avoid detection by predators, ability to recognize predators, and stamina to flee from predators. Techniques presently exist for improving each of these antipredator attributes of cultured fish

Cryptic Coloration

Postrelease survival of cultured fish can be increased by rearing them in an environment that promotes full development of the camouflage pattern they will need after release. Both the short- and long-term camouflage coloration of salmonids is primarily affected by the background color pattern of their environment. Short-term physiological color changes are accomplished by chromatophore expansion: pigment is dispersed within the chromatophore unit and color change occurs within minutes. In contrast, morphological color changes take weeks to complete as pigments and chromatophore units are developed to match the general background coloration (Fuji 1993). The cryptic coloration ability generated by these long-term stable color adaptations provides the greatest benefit for avoiding detection by predators.

Fish culturists have long recognized that fish reared in earthen-bottom ponds have better coloration than those reared in concrete vessels (Piper et al. 1982). However, only recently has it been understood that rearing salmonids over natural substrates, similar to those over which they will be released, increases postrelease survival by enhancing cryptic coloration. Groups of brook trout reared for 11 weeks over distinct background colors were less vulnerable to predators when challenged over background colors similar to those over which they were reared (Donnelly and Whoriskey 1991).

In our laboratory, fall chinook salmon reared in seminatural rectangular tanks with substrate, cover, and instream structure (plants and rootwads) had better cryptic coloration for the stream environment into which they were released than did fish reared in barren grey tanks similar to the surroundings in conventional raceways. These seminaturally reared fish had almost 50% higher postrelease survival in a coastal stream than their conventionally reared counterparts (Fig. 3-l). As there was no observed difference in size or disease status between the treatments, the difference in survival is probably attributable to coloration.

Similar relationships have been noted by other investigators. In one coho salmon enhancement project by the Lummi Indian Nation, fish reared in dirt-bottom ponds had higher smolt-to-adult survival than those reared in concrete vessels (K. Johnson, Idaho Department of Fish and Game, pers. commun., 1992). Besides having better cryptic coloration, fish reared in earthen ponds are considered to have better health, fin condition, and overall quality than those reared in concrete vessels (Piper et al. 1982). This was recently verified by Parker et al. (1990) in a study that demonstrated that coho salmon fry teared over leaf litter had higher prerelease survival than those reared in barren-bottom tanks.

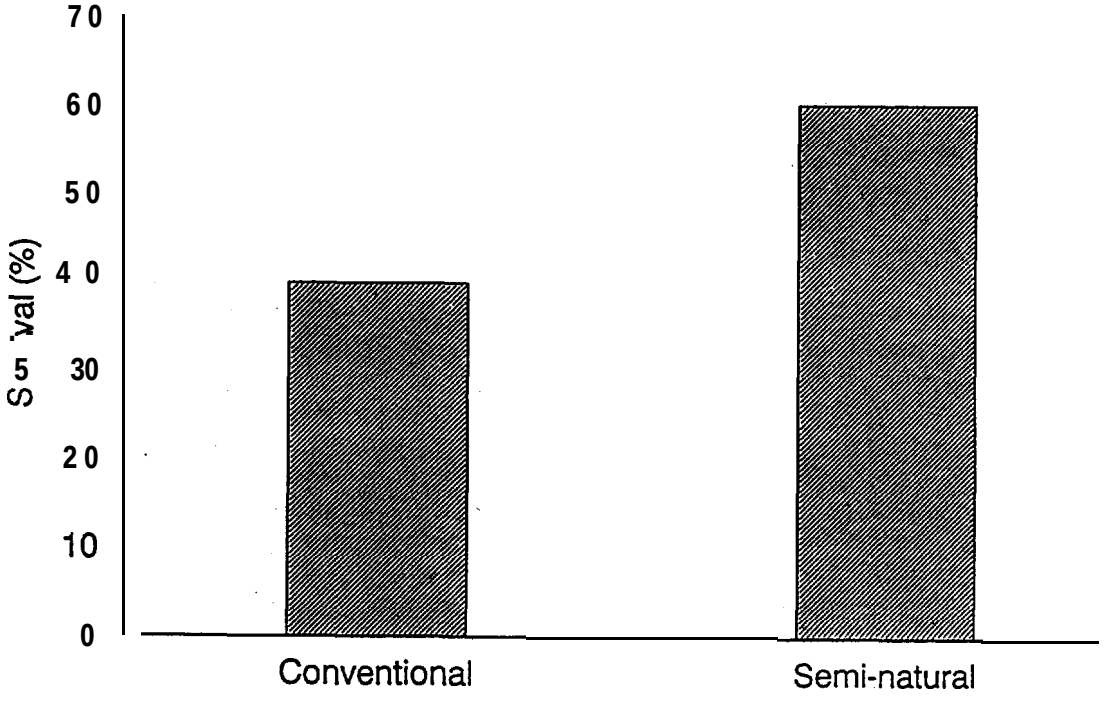

Figure 3-l. Instream survival of fall chinook salmon released from conventional (barren; n = 83) and seminatural (substrate, structure, cover, n = 203) raceways.

Predator Avoidance

Postrelease survival of cultured salmonids can also be increased by training them to recognize and avoid predators. Thompson (1966) first determined that salmonids can learn to avoid predators in the laboratory and then demonstrated that predator avoidance training is practical in production hatcheries. He conditioned production lots of fall chinook salmon to avoid predators by moving an electrified model of a predacious trout through raceways each day for several weeks. Salmon that approached the model too closely were negatively conditioned with an electrical shock. After they were released into a coastal creek, the instream survival of the salmon trained to avoid predators was significantly higher than that of their untrained cohorts.

In the laboratory, it has been shown that coho salmon rapidly learn to recognize and avoid a predator after observing it attack conspecifics (Olla and Davis 1989). This approach to predator-avoidance training could be implemented by briefly exposing each lot of production fish to the main predators they will encounter after release. The loss of a few fish sacrificed in these training sessions should be outweighed by the larger number of trained fish that may survive later.

Swimming Performance

Swimming ability, which is critical to a fish's ability to escape from a predator, can be improved by implementing exercise programs. The swimming performance of coho salmon, Atlantic salmon, and brook trout significantly improved after they were forced to swim at higher velocities for 6 weeks or more (Besner and Smith 1983, Leon 1986, Schurov et al. 1986a). This exercise regime also enhanced their growth. The postrelease survival of exercised fish has generally (Burrows 1969, Wendt and Saunders 1972, Cresswell and Williams 1983, Leon 1986, Schurov et al. 1986b), but not always (Lagasse et al. 1980, Evenson and Ewing 1993), been higher than that of unexercised fish. The survival benefit of exercise was only realized in programs that forced salmonids to swim at high velocities for some time each day for at least 2 weeks. This exercise training may be implemented with present technology by rearing fish in either circular or rectangular high-velocity circulating-water ponds or by creating high velocities in conventional raceways by temporarily drawing them down or recirculating water within.

Foraging Training

Foraging theory suggests that supplementing standard pelletized diets with live foods will profoundly increase postrelease foraging ability of cultured fish. Gillen et al. (198 1) found that previous experience in capturing Jive prey enhanced the foraging behavior of tiger muskellunge (F_1 hybrid of female muskellunge, Esox *masquinongy*, and male northern pike, E. *lucius*) by decreasing the time and number of strikes required to capture natural live prey.

In our laboratory, fall chinook salmon reared on a pellet diet supplemented with live prey fed on twice as many familiar (e.g., chironomid larvae) and novel prey (e.g., mayfly larvae) as their counterparts reared on a pellet-only diet (Fig. 3-2). Even though food was abundantly supplied to both treatment groups, the growth of fish reared on the live-food supplemented diet was greater than that of fish fed only pellets.

Field trials generally confirm that live-food supplemented diets improve the postrelease foraging ability and survival of cultured fish. Tiger muskellunge reared in the hatchery on a live fish diet had higher postrelease survival than their cohorts reared only on pellets (Johnson 1978).

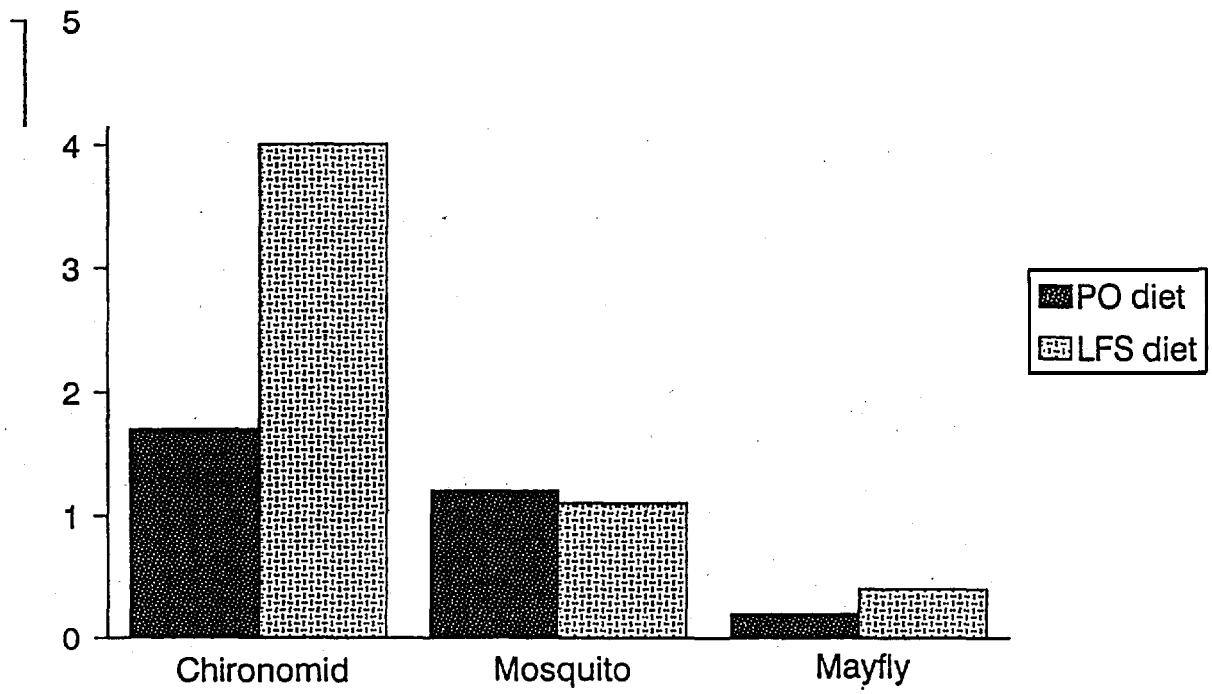

Figure 3-2. Average number of prey ingested by fall chinook salmon reared on pellet-only (PO, n = 20) or live-food supplemented (LFS; n = 20) diets.

Similarly, brown trout reared in earthen-bottom ponds with natural food supplementation had a higher postrelease survival than did control trout reared in non-earthen-bottom tanks and fed only commercial pellet diets (Hesthagen and Johnsen 1989). Live foods for salmonids can be produced by adopting techniques used in the culture of many warmwater fish species. Besides the beneficial effects on fish, live food diets have the potential to both reduce feed costs and produce less undigested waste than standard diets.

Supplemental Dissolved Oxygen

The level of dissolved oxygen in the rearing environment is critical for salmonids. At rest, a fish uses up to 10% of its metabolic energy to support gill ventilation (Wooten 1990). If the oxygen content of water declines, available energy must be directed from other life functions to increase respiratory ventilation. The difference between the energy required for respiration and the total available energy is the metabolic scope for activity.

At 15°C, salmonids require 10 mg/L of dissolved oxygen to be fully active (McCauley 1991). A brook trout living in water with 7 mg/L dissolved oxygen has only three-fourths of the metabolic scope of a trout living in water with 10 mg/L dissolved oxygen (Fry 1971). Thus, although salmonids can survive and grow in a 7-mg/L dissolved oxygen environment, their metabolic scope is sharply curtailed.

As the metabolic scope for activity is reduced by lower levels of available dissolved oxygen, there is a commensurate decrease in activities such as sustained swimming performance. Growth and food conversion are also limited by available dissolved oxygen. In a study using coho salmon, Herman et al. (1962) showed that growth and food-conversion efficiency increased with a rise in environmental dissolved oxygen up to the highest level tested (8.3 mg/L). Theoretically, both learning ability and disease resistance of fish may similarly be limited by dissolved oxygen.

Fish culture textbooks suggest that a 7-mg/L-dissolved-oxygen environment is satisfactory for rearing salmonids and that the dissolved oxygen level should never fall below 5 mg/L (Leitritz and Lewis 1980, Piper et al. 1982, Mclamey 1984). However, these texts also indicate that higher dissolved oxygen levels are preferred for improving fish quality and reducing stress. Piper et al. (1982) indicate inflow water to ponds should be at 100% oxygen saturation and never drop below 80% oxygen saturation anywhere in the pond. Leitritz and Lewis (1980) indicate that a 10 to 1 l-m&-dissolved-oxygen environment is best for culturing trout, which may show discomfort at a level of 7.8 mg/L. The recommended 10 to 1 1-mg/L-dissolved-oxygen level should provide salmonids with a full metabolic scope of activity.

A 10 mg/L dissolved oxygen environment can be achieved in the fish culture environment with supplementation oxygen technology. Most research on this technology has been used to increase the weight of fish that can be produced per unit volume (Dwyer et al. 1991). However, it has also been observed that in hatcheries utilizing oxygen injection and supplemental aeration systems, disease incidence decreased and fin quality, feed conversion, and fish survival improved (Marking 1987). The cost and inconvenience of retrofitting these systems to production hatcheries is relatively low compared with the benefits in fish quality that can be achieved.

Rearing Density

Rearing density is one of the most important and well-studied factors affecting fish quality. In rainbow trout (0. *mykiss*) both growth and condition factor are inversely related to rearing density (Refstie 1977). Westers and Copeland (1973) and Maheshkumar (1985) found that the fin condition of Atlantic salmon deteriorated with increasing rearing densities. However, in a study in which another strain of Atlantic salmon was reared in a different type of vessel at rearing densities of 8.5 to 68.7 kg/m^3 no relationship between rearing density and fin condition, growth, or in-culture survival was found (Soderberg and Meade 1987).

Inverse relationships between rearing density and growth, condition factor, and food conversion efficiency have been observed in coho salmon (Fagerlund et al. 1981). In addition, coho salmon reared at high densities suffered greater physiological stress as measured by body water content, fat and protein contents, interrenal cell nuclear diameter, and mortality rates. For coho salmon smolts, rearing densities as low as 16 kg/m^3 (1 lb/ft^3) can induce physiological stress (Wedemeyer 1976), and increased rearing density reduces both gill ATPase levels (Banks 1992) and plasma thyroid hormones (Pitano et al. 1986).

In a survey of 85 variables related to strain and culture conditions, only the five associated with either water flow, amount of living space, or relative water level in rivers explained the postrelease survival of Atlantic salmon (Homer et al. 1979). The adult return of coho salmon also appears to be inversely related to rearing-pond density in some (Sandercock and Stone, unpublished,-as reported in Fagerlund et al. 1981; Banks 1992), but not all, studies (Hopley et al. 1993).

Martin and Wertheimer (1989) examined the effect of one low, two intermediate, and one high rearing densities on the postrelease survival of chinook salmon. In the hatchery, all four rearing densities showed similar high survival (99.5% or greater), but fish reared at higher densities were smaller at release. The low-density group showed the highest adult return (1.0%), followed by the two intermediate-density groups (0.9 and 0.7%) and the high-density group (0.6%). However, the increased number of smolts produced at the two higher densities compensated for their reduced return rate and yielded a higher number of adult returns per unit volume of rearing space.

Most other chinook salmon studies have shown a consistent inverse relationship between rearing density and percentage of fish surviving to recruit to the fishery and spawning area (Hopley 1980, Fagerlund et al. 1987, Denton 1988, Downey et al. 1988, Banks 1990). However, as adult return is a function of both the number of fish released and the percentage of that number surviving to adulthood, the greatest number of fall chinook adults can be produced by rearing fish at intermediate densities (Martin and Wertheimer 1989).

The relationship between rearing density and adult returns for all salmonid species indicates that a larger percentage of fish recruit to the fishery and spawning population when they are reared at a lower density. Thus, for any given number of cultured juveniles, the total adult yield will be greatest when they ate reared in a large (low density) rather than a small (high density) volume vessel; Because water, not land, is the primary constraint at most fish-culture facilities, postrelease survival and total adult returns can be increased'by installing larger vessels that reduce density by increasing rearing volume.

Conclusions

As demonstrated in this review, there are many culture strategies for increasing the postrelease survival of hatchery-reared salmonids. Strategies that involve rearing salmonids at low densities with naturalistic substrate, instream structure, and cover should reduce chronic stress and disease, and increase survival. These strategies should also minimize potential risks from the shifts in selection pressures associated with the conventional culture environment. Strategies such as foraging training, swimming exercise, and antipredator conditioning should also behaviorally and morphologically prepare fish for survival in the postrelease environment.

Traditionally, these strategies have been rejected by hatcheries because it has been presumed that they will increase costs, maintenance, or disease. These concerns are either unfounded or can be eliminated with alternative technology. For example, salmonids can be reared at a lower density over natural substrates in large dirt-bottom raceways or ponds without increasing water consumption or incurring the higher construction costs associated with concrete ponds. Similarly, the harvest of natural feeds from on-site production facilities will enhance foraging ability and overall fish quality. Natural feeds may also reduce overall feed costs and enhance effluent water quality by reducing the generation of undigested settleable solids.

Culture strategies that increase postrelease survival can significantly reduce salmon enhancement costs. Based on several sources, we estimated the traditional cost per smolt at publicly operated facilities at about US$0.15 for coho salmon, $0.25 for spring chinook salmon, and $0.34 for steelhead (Mayo 1988; Heen 1993; R. Hager, Hatchery Consultants, Inc., pers. commun. 1994). The quantity of smolts an enhancement program must produce to yield a given number of recruits is dependent on the smolt-to-adult survival. Thus, culture strategies that increase smolt-to-adult survival reduce both the total number of smolts a program must release and the cost per recruit. For example, for a spring chinook salmon smolt costing $0.25 to produce, doubling postrelease survival from 0.5 to 1.0% reduces production costs for each recruit from $50 to $25 for a net saving of $25 per recruit. For enhancement programs designed to produce half a million recruits, implementation of these culture strategies could save up to $12 million in smolt production costs each year.

There are also significant benefits to the natural spawning population that arise from increasing the postrelease survival of cultured fish. For instance, doubling postrelease survival from 0.5 to 1.0% could reduce the number of adults that culture programs must remove from wild-spawning populations by half. This reduction in the number of broodstock required is crucial for conservation and supplementation programs designed to build naturally spawning populations, as well as for enhancement facilities that are mining naturally spawning populations for broodstock. This increase in postrelease survival also halves the number of hatchery fish that must be released to produce a given number of recruits. This should reduce the postrelease competition for resources that occurs between wild and hatchery fish, thus potentially improving wild fish survival. These culture strategies may also minimize the genetic impact of cultured fish spawning with the natural population by inhibiting the development of domestic strains that are distinct from the wild strains from which they were derived. Finally, by producing fewer smolts, enhancement facilities will produce less biowaste and use less natural resources than they do with traditional fish culture practices.

In summary, the reviewed innovative culture strategies could benefit wild stocks as well as target cultured salmonids by reducing broodstock collection and smolt release numbers and by lessening domestication and environmental impacts.

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Section 4

THE EFFECTIVENESS OF LIVE FOOD SUPPLEMENTATION IN IMPROVING THE FORAGING ABILITY OF FALL CHINOOK SALMON, 1992[2]

by

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Contents

Introduction

The low postrelease survival of cultured salmonids used in enhancement and supplementation may be partially due to their inability to forage on naturally available foods (Miller 1952, Hochachka 1961, Reimers 1963). It is generally recognized that during the first weeks after release, cultured salmonids eat less and forage on more inedible material than wild fish (Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986).

This difference in foraging may result from the following causes: 1) stress associated with entering a new environment; 2) the inability of pellet-reared fish to recognize live food; 3) taste bias against live food developed in pellet-reared fish; or 4) the inability of pellet-reared fish to develop successful hunting tactics.

Stress associated with entering a new environment may be reduced by rearing fish under seminatural conditions. In addition, postrelease foraging may be improved by training fish to feed on live food in the hatchery. This study compared the foraging ability of fall chinook salmon reared on pelletized feed to that of fish reared on a combination of pellets and live food.

Methods

The research was conducted at the National Marine Fisheries Service (NMFS) Freshwater Fish Culture Laboratory at the University of Washington's Big Beef Creek Research Station near Seabeck, Washington. The facility is adjacent to the estuary of Big Beef Creek, a small coastal stream.

Age-O fall chinook salmon (*Oncorhynchus tshawytscha*) fry were obtained from the Washington State Department of Fisheries George Adams Salmon Hatchery and were acclimated to the NMFS laboratory for 2 months prior to the initiation of experimental rearing. These fish were fed commercially available pelletized diets from swimup (February 1992) until April 1992, when they were measured and randomly dispersed among six 2-m-diameter blue polyethylene tanks. Each tank received 150 fry and was supplied with clear 10°C well-water. Fish in three of the six tanks were fed commercially available pellets only (PO), while those in the other three tanks were fed a pellet diet supplemented with live food (LFS).

Fish were reared under these experimental conditions for 3 months. Every morning, fry in the three LFS tanks were presented with various combinations of live food (mysids, chironomid larvae, mosquito larvae, and daphnia that are referred to as "familiar" prey). After 1 hour, these fish were fed to satiation with a pelletized ration. Fry in the three PO-diet tanks were fed to satiation on pellets only. Both groups were fed to satiation in an attempt to equalize utilizable energy intake and growth between the two treatment groups. Except for their diets, both groups were cultured using the same general procedures outlined by Leitriz and Lewis (1980).

The live foods used in the study were either cultured on site, following the general methods outlined by Masters (1975), or harvested from an adjacent stream. The daphnia, chironomid larvae, and mosquito larvae were grown in fertilizer-enriched water in several 2-m-diameter by 0.3-m-deeppolyethylene swimming pools. Burlap sacks were added to each pool to provide suitable substrate for the chimnomids. Daphnia were seeded into the pools from a stock population while the chironomid and mosquito larvae were naturally recruited to the pools from the local population. The mysids were harvested with an aquarium net from the Big Beef Creek estuary at high tide just below the stream weir. Mayfly larvae, which were subsequently used as

novel prey, were harvested from the stream by overturning submerged stones and collecting the disturbed larvae in a small, fine-mesh seine.

On 15 July 1992, all fish were anesthetized in tricainemethane sulfonate, weighed, measured, and visually examined for coloration and fin condition. A subsample was divided into three length classes and maintained separately in 400-L aquaria for use in foraging effectiveness evaluations. A second subsample of fish (PO n = 42, LFS n = 35) was sacrificed and examined for bacterial kidney disease (BKD) to determine if live food supplemented diets affected the incidence of this common chinook salmon pathogen.

Foraging effectiveness was evaluated by comparing the foraging behavior of fish subsampled from the LFS and PO treatments under controlled laboratory conditions. Foraging behavior was observed in a barren, 200-L, acrylic aquarium 91cm long by 38-cm wide by 51-cm deep, with an opaque background on all but the front side. A total of 40 trials were conducted in this test aquarium. For each trial, a single fish from one of the two treatments was allowed a minimum of 60 min to acclimate to the new aquarium Fish were then allowed to forage on mosquito, chironomid, and mayfly larvae by introducing all prey simuhaneously into the test arena. Each trial lasted 30 min. Fish from the two treatment groups were alternated between trials until a minimum of 20 fish from each treatment had been examined in the test aquarium.

An observer used event recorder software on a personal computer to record the species of prey interacted with as well as the time of approach (swimming in general direction of prey), attack (burst swimming toward prey), capture, ingestion, or loss or rejection of each prey item. Temporal foraging efficiency was calculated from the average prey handling time (from attack to ingestion) of fish from each treatment. Foraging success was determined by the average number of prey of each type approached, attacked, captured, and ingested by each fish.

The prevalence of BKD was determined with standard fluorescent antibody technique (Bullock and Stockey 1975). The differences in length, weight, and temporal aspects of foraging efficiency between treatments were analyzed with Student's t-tests. The approach, attack, capture, and ingestion data were analyzed with Mann-Whitney U tests (Zar 1974).

Results and Discussion

Prey Behavior

The interaction between predator and prey differed markedly betweenprey type. Mosquito larvae appeared to avoid predation by remaining motionless at the surface. Those few that swam down from the surface usually attracted the attention of the fish and were readily attacked and ingested whole. In contrast, the wriggling bright red chironomid larvae were usually attacked by any fish that spied them on the bottom. In many cases, the fish would ingest several of these worm-shaped insects in a single attack. In the test aquarium, chironomid larvae did not appear to have any antipredator strategy.

The relatively large, heavily armored, and dorso-ventrally flattened mayfly larvae were the most difficult prey for the fish to handle. The fish had to tear each mayfly larva into pieces and ingest the smaller portions. Interestingly, after ingesting one mayfly larva, fish were usually. reluctant to ingest another, even though they continued to approach and attack these insects. This high rate of rejection after the initial mayfly larva was eaten suggests this particular mayfly species may have been unpalatable. A second antipredator strategy observed in mayflies was to remain

motionless whenever any mayfly in the tank was attacked. This strategy was successful against visually-hunting predators like salmonids, for which the primary cue that releases prey-attack behavior is movement within their visual field

Foraging

The fish from the LFS tanks ingested twice as many and significantly (P = 0.032) more chironomid and nonsignificantly (P = 0.3%) more mayfly larvae as fish from the PO tanks, whereas fish from both treatment groups ate similar numbers of mosquito larvae (P = 0.796) (Fig. 4-l). In general, all other major classes of foraging behavior (approach, attack, and capture) on chironomids and mayfly larvae were higher for LPS-treatment fish than PO-treatment fish (Pig. 4-2). However, the differences were only statistically significant ($P \leq 0.05$) in number of prey attacked, captured, and ingested for chironomid larvae. Since LFS fish were noticeably more bottom oriented than PO fish, it is not surprising that they attacked and ingested more chironomids. This orientation may have been conditioned by their foraging on the bottom for chironomids during the live-food supplementation phase of the experiment.

Twenty-five percent of the LFS-treatment fish and 40% of the PO-treatment fish failed to attack prey. This is similar to Paszkowski and Olla's (1985) findings that many hatchery coho salmon (0. *kisutch*) would not feed in test arenas. They attributed this to handling stress, rather than a rejection of live prey. However, the difference observed between treatments in the present study suggests fish reared on pellets may not have developed the ability to recognize live prey as food. Bryan and Larkin (1972), Ringler (1985), and Merna (1986) reported that juvenile salmonids can develop initial food preferences that are maintained throughout life. Therefore, to be fully effective, live-food supplementation training may need to be initiated at the swimup stage.

More effective foraging on both familiar and unfamiliar prey by experienced fish suggests that fish can generalize their experience with live food, however novel the prey. This is crucial if live-food supplementation is to enhance the postrelease foraging ability of migratory species, which will encounter a wide variety of prey species in nature. Furthermore, it suggests that even if individual fish develop early and narrow preferences, they can switch to other forms of live prey once they are weaned off pellets.

Prior exposure to live food appeared not to enhance foraging efficiency (Fig. 4-3). To increase the foraging efficiency of cultured fish, we may need to train them to forage on more complex prey and in more structurally complex environments.

Morphology

Although fish in both treatments were fed to satiation, fish in the LFS tanks were significantly ($P \leq 0.05$) longer and heavier than those in the PO group (Table 1). This may have resulted from their having more opportunities to feed during the day, more total nourishment available, or live food containing essential trace elements or vitamins not present in sufficient quantity in the pellet diets. Withii the confines of this study there is no conclusive way to isolate these factors.

There were no obvious differences in coloration or fin condition between fall chinook salmon in either treatment. In contrast, in a previous study cutthroat trout (0. *clarki*) reared exclusively on live food had noticeably better coloration and fin condition than those reared on pellet-only diets (personal observations). While preliminary, this suggests that live-food supplementation does not provide the enhanced coloration and better fin condition associated with

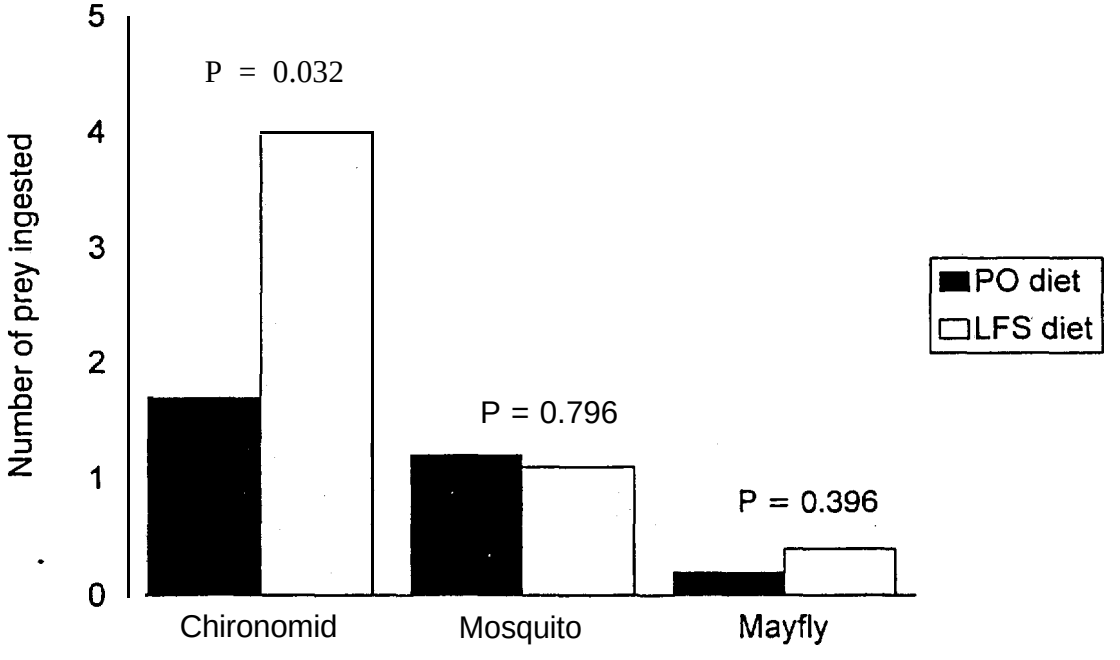

Figure 4-l. Average number of test prey ingested by fall chinook salmon reared on pellet-only (PO; n = 20) or live-food-supplemented (LFS; n = 20) diets. Probability values based on Mann-Whitney U tests.

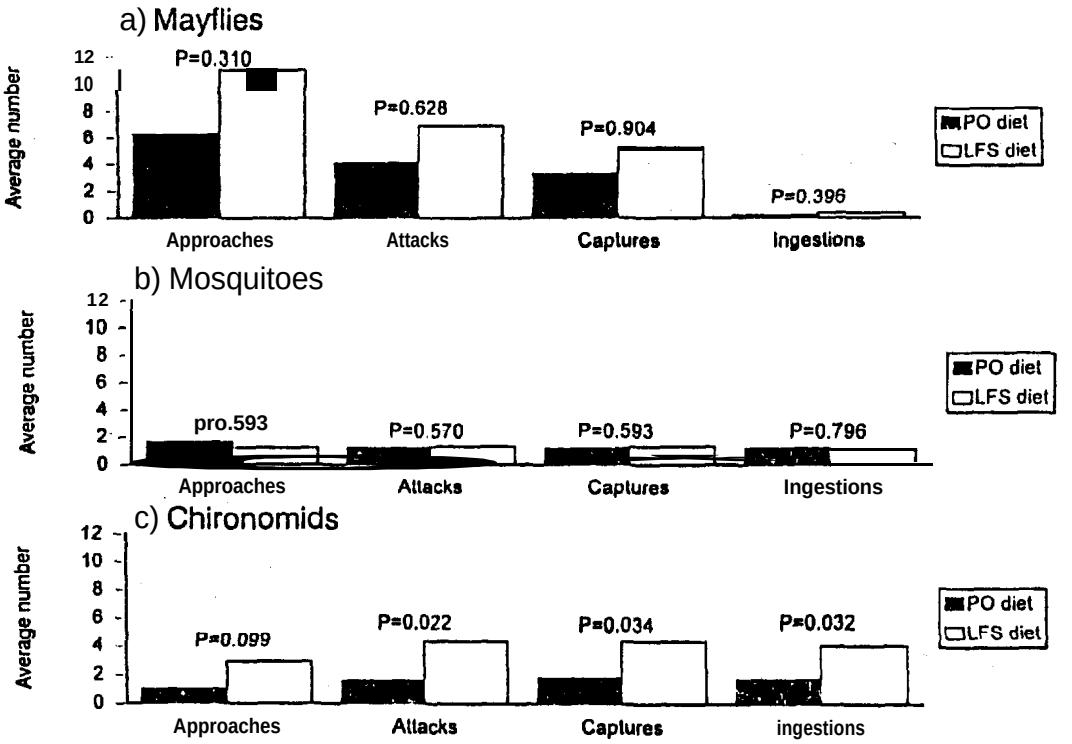

Figure 4-2. Foraging behavior on a) mayflies, b) mosquitoes, c) chironomids by fall chinook salmon reared on pellet-only (PO; n = 20) or live-food-supplemented (LFS; n = 20) diets. Probability values based on Mann-Whitney U tests.

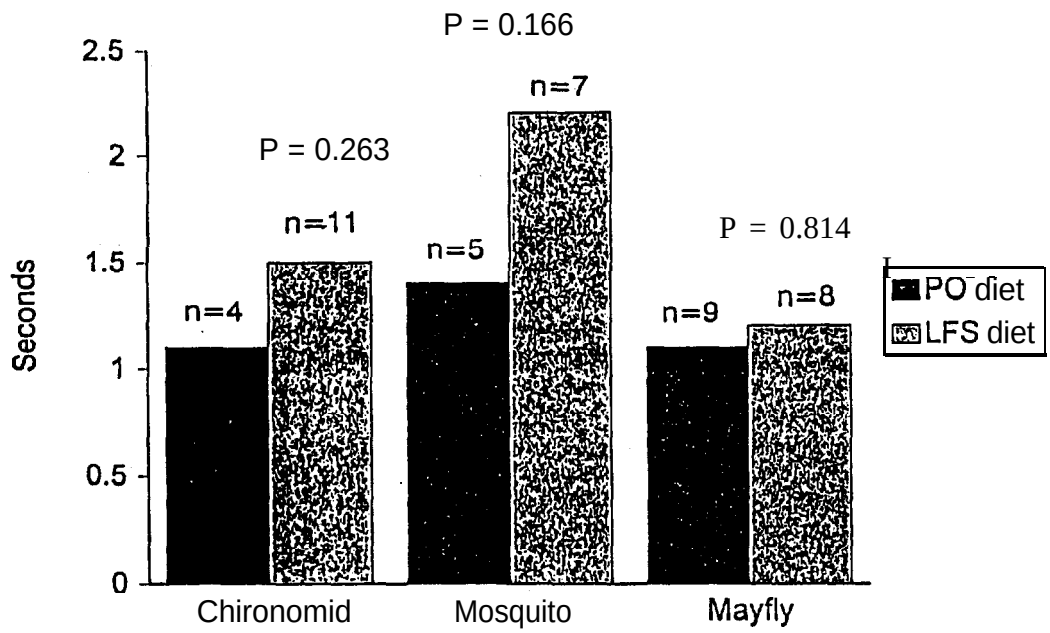

Figure 4-3. Foraging efficiency (average handling time) of fall chinook salmon reared on pellet -only (PO) or live-food-supplemented (LFS) diets. Probability values based on t-tests with n being determined by the number of fish that ate at least one of the prey.

Table 4-l. Comparison of length and weight of fall chinook salmon reared on commercially pelletized diets with and without live food supplements.

Variable	Treatment diet: Pelletized ration	Treatment diet: Pelletized ration plus live-food supplement
Number	446	449
Length (mm)		
mean	109.5*	111.2*
SD	6.9	7.1
Weight (g)		
mean	16.4*	17.4*
SD	3.4	3.6

* Significantly different at $P \leq 0.05$.

total live food diets, or that them are species-specific differences in how diet interacts with coloration and fin condition.

Disease Analysis

There was no significant difference in the incidence of BKD in fish from either treatment. At subsampling, no evidence of BKD was found in either treatment group.

Conclusions

The findings of this and other studies (Johnson 1978, Hesthagen and Johnsen 1989) suggest diets supplemented with live food may enhance the postrelease foraging ability and survival of cultured fish used in enhancement and supplementation. Future work should concentrate on exposing fish to difficult to handle prey in semi-natural structured habitats. Implementation of this technique, along with other life-skill training approaches (Suboski and Templeton 1989), such as antipredator training (Thompson 1966, Olla and Davis 1989), offers the possibility for dramatically improved postrelease survival of cultured fish.

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Section 5

THE BEHAVIOR AND POSTRELEASE SURVIVAL OF FALL CHINOOK SALMON REARED IN CONVENTIONAL AND SEMINATURAL RACEWAYS, 1992[3]

by

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Contents

Introduction

In 1992, we constructed a rearing environment (for chinook salmon) comprised of sand and gravel substrates, aquatic plants for instream structure, and overhanging cover. We theorized that salmonids cultured in raceways that simulated their natural environment should develop more natural behavior and cryptic coloration, and should have higher rates of postrelease survival than those reared in conventional raceways. The initial experiment described in this section compared the effect of this seminatural rearing environment vs. a conventional culture environment on the behavior, coloration, disease status, growth, and postrelease survival of fall chinook salmon (*Oncorhynchus tshawytscha*).

Material and Methods

This study was conducted at the National Marine Fisheries Service (NMFS) Freshwater Fish Culture Laboratory at the University of Washington's (UW) Big Beef Creek Research Station near Seabeck, Washington. Fall chinook salmon eggs were obtained from the UW Big Beef Creek Hatchery.

Fish were reared under one of three treatment conditions (Fig. 5-1). The conventional treatment represented a standard raceway environment (as described by Leitritz and Lewis 1980, Riper et al. 1982), and thus lacked any substrate, structure, or opaque overhead cover. The other two treatments represented seminatural rearing environments, with plastic aquarium plants and live watercress mot wads for instream structure and with opaque covers to simulate overhanging banks. Seminatural rearing treatments were outfitted with either sand or undergravel filter covered with pea gravel on the tank bottom. Four rearing tanks were used per treatment, and fish in all three treatments were fed a standard prepared pellet diet from the surface by hand. No therapeutic treatments were required during the study.

Fish were reared in 12 rectangular 400-L acrylic tanks. Each tank was 46 cm wide, 46 cm deep, 152 cm long, and was maintained at a depth of 43 cm Four liters per minute of 10°C well water was supplied to each tank through a lo-cm-diameter opening. All 12 tanks were supplied with air via four airstones spread along the bottom rear of each tank. Sheets of grey-black painted polystyrene were fitted to the outside of both ends, the rear, and the bottom of all tanks to simulate the grey concrete background coloration of a standard raceway. The experimental rearing vessels were set up outside in two banks of six tanks each. The area between banks was enclosed in a tent, which darkened the area and thus enabled observers to watch the fish without disturbing them. The tanks were lit from the surface by ambient sunlight. Each week, algae was scrubbed off the sides of the tank and flocculent material siphoned off the bottom.

The rearing experiment was initiated by randomly dividing a population of 480 Big Beef Creek hatchery fall chinook salmon swimup fry among the 12 model raceway tanks (i.e., 40 fry/tank). Each fry was anesthetized with MS-222 and measured to the nearest mm.

Aggression and foraging activity were the only variables measured during the experimental rearing period. Nipping and debris-striking were estimated by observing fish behavior in each tank for a 10-minute period each week The observer scanned each tank until observing either type of activity, and then recorded the behavior and resumed scanning the tank for new activity.

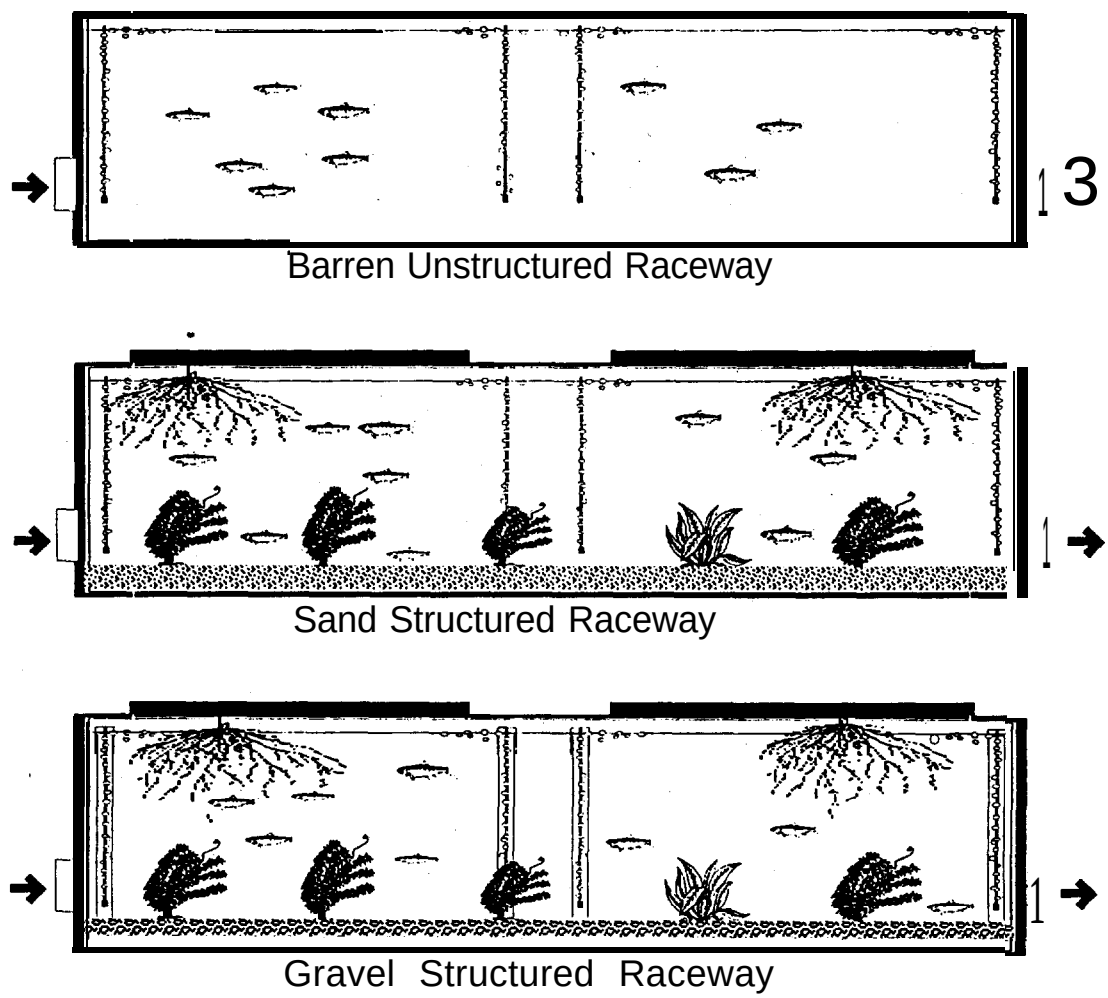

Figure 5-l. Unstructured conventional (Barren) and structured seminatural (Gravel, Sand) raceway habitats that fall chinook salmon were reared in at the Big Beef Creek Facility near Seabeck, Washington, 1992.

Nipping behavior included all contact nips, threat nips, and miss nips as defined by Maynard (1987). Foraging strikes refer to attacks on air bubbles, decaying food, and fecal debris. No food was presented to fish on observation days until after the observation period.

Experimental rearing was terminated on 20 May 1992 after all the fish experienced a transient color change that indicated they had undergone their first smoltification. All the study fish were anesthetized with MS-222, measured, and weighed, and every fourth fish was euthanatized in a lethal concentration of MS-222 for cryptic coloration and pathological analyses. The anesthesia eliminated neural control of chmmatophore units, assuring that the observed color. differences represented cell structure changes rather than behavioral differences. The majority of fish were tagged with passive integrated transponder (PIT) tags, following the method described by Prentice et al. (1991), and used to evaluate the effect of the rearing treatments on postrelease survival.

Each fish in the lethal subsample was submerged on its side in a shallow tray filled with water and was then photographed under standardized lighting conditions. Resulting photographic slides were viewed on a video monitor, and the images were analyzed visually and with the aid of computer software. The base skin color immediately below the dorsal fin and above the lateral line was matched to color chips from Ma&z and Paul (1950). Brightness, chrome, and hue of each color chip was then determined with a colorimeter, and the relative darkness of each parr mark was visually matched to chips on a Kodak gray scale. The length and width of each of the three anterior-most parr marks was measured and used to calculate parr mark area. This number was divided by total body area (fork length multiplied by width) to determine relative parr marked area. The number of observable lesions on the photographed fish were counted. In addition, each of the visible fins of the photographed fish were examined for fraying and evidence of erosion.

Euthanatized fish were dissected and examined for the presence of bacterial disease organisms in the kidney. A sterile inoculation loop was first dipped into the kidney and then streaked across prepared media in a petri dish. Petri dishes were incubated at room temperature and after 24 hours examined for the presence of furunculosis (Aeromonas *salmonicida*) or enteric redmouth (Yersinia *ruckeri*) organisms. After plating, the kidney was removed and homogenized. A clean cotton swab was then used to streak homogenized kidney across glass slides that were then examined for the presence of Renibacterium *salmoninarum*, the causative agent of bacterial kidney disease (BKD), using the florescent antibody technique presented by Bullock and Stockey (1975).

Tagged fish were held in a 2,000-L fish transport tank overnight and then released into Anderson Creek near Seabeck, Washington, where they were challenged to survive at 2.1 km outmigration to an estuarine weir. Anderson Creek is a small coastal stream with a heavily wooded riparian zone that supports a healthy population of cutthroat trout (0. *clarki*), rainbow trout (0. *mykiss*), and coho salmon (0. *kisutch*), but lacks a chinook salmon run. The outmigration was monitored at the weir for more than 30 days. At the end of the study, the majority of the creek was electrofished to ensure that the study fish had not taken up residence within the creek.

Results and Discussion

Growth and Survival during Rearing

There were no significant differences ($P > 0.05$) in length or weight of fish reared in any of the three treatments (Table 5-1). The condition factor (ratio of length/weight, as described in Piper et al. 1982) was similar for all three treatments, and prerelease survival was nearly 100% for all replicates in both treatments. However, a few fish died from jumping out of the tanks in conventional treatment groups.

No disease-related mortalities occurred, and no fin fraying or erosion was noted in fish from any of the three treatments. However, fish in both seminatural treatments had more skin lesions (15.4%) than fish in the conventional treatment tanks (5.6%). Kidney tissue cultures indicated neither furunculosis, enteric redmouth, nor BKD was present in any of the treatment groups. However, unidentified diplococcus bacteria and yeast were found significantly more often ($P = 0.003$) in cultures taken from fish reared in the sand-bottom tanks than from fish in the other two treatments. It is possible that bacteria were introduced into the structured treatment tanks with the watercress or sand. We recommend that in future experiments, all inorganic material be sterilized to reduce the risk of disease contamination from these sources.

Raceway Maintenance

Both the conventional and seminatural tanks used in this study appeared to foul mote quickly than other raceway systems we have worked with. The growth of filamentous algae on plastic aquaria plants was the most difficult to clean. It was also observed that chinook salmon failed to feed on pellets which had fallen over pea-gravel, but did retrieve feed from the floor of sand or acrylic bottom tanks. However, a prototype seminatural raceway containing sand substrate and a sheared 2-m-tall Douglas fir (*Pseudotsuga* menziesii) for structure, presented no unusual cleaning problems (D. Maynard, NMFS, pers. observation). Therefore, we suggest that large-scale seminatural rearing efforts focus on using sand substrates layered over pea-gravel-covered undergravel filters. Sheared live trees should also be used to provide instream structure rather than plastic foliage.

Fish Behavior

Fish in both types of seminatural tanks exhibited significantly greater ($P = 0.046$) aggressive activity (contact nips, threat nips, and chases) than fish in unstructured tanks. Aggressive activity did not significantly differ ($P = 0.096$) between the two structured treatments. Subordinate chinook salmon in the seminatural tank often sought refuge from aggression in the watercress root wads. The plastic aquarium plants may have provided territorial focal points for dominant individuals, as has been observed in other studies (D. Maynard, NMFS, unpublished data). The greater frequency of aggression in the tanks containing structured habitat may be related to the greater number of territorial focal points afforded by structure:

Fish in conventional rearing habitats exhibited a significantly greater ($P = 0.004$) number of debris strikes than those reared in either of the seminatural tanks. Since these fish were not fed prior to or during observations, foraging was primarily directed at decaying food and fecal material drifting in the water column. Organic debris (particularly fecal material) is considered a potential source of horizontal disease transmission in cultured fish. Lower levels of debris within the water column of seminatural tanks may have been responsible for the reduced foraging activity recorded in these two treatments. Apparently, either the plants, substrates, or interstitial microorganisms

Table 5-1. Number, length, weight, and condition factor[a] of Big Beef Creek fall chinook salmon reared in three artificial habitats, 1992.

	Habitat type		
	Unstructured	Structured	
Variable	Barren	Gravel	Sand
Length (mm)[b]			
n	138	154	154
Mean	74.5	74.3	74.8
sd	7.6	4.9	5.1
Weight (g)[b]			
n	138	154	154
Mean	4.4	4.1	4.3
sd	1.4	1.0	1.0
Condition factor[b]			
n	138	154	154
Mean	1.01	0.99	1.01
sd	0.05	0.05	0.05

[a] Condition factor = weight (g)* 100/length (cm)^3

[b] There were no statistical differences ($P < 0.05$) between treatments based on ANOVA.

present in seminatural tanks removed these organic particles from the water column: this suggests that structured habitats may provide a more sanitary rearing environment than unstructured tanks.

Morphology

No significant difference was detected in the number of dorsal spots developed by fish in any of the three treatments. This supported the hypothesis that spotting pattern is primarily under genetic rather than environmental control. Following the logic of Donnelly and Dill (1984). sahnonid spot patterns may differ between stocks, with each stock evolving a pattern that matches the grain of its native habitat,

The base integument coloration of fish from all three treatments had a similar brightness component, but the chroma and hue of seminaturally reared salmon was significantly different from that of conventionally reared fish (Table 5-2). The grey scale rank of parr marks was similar for fish from all three treatments.

Subjective observations, made over the last 2 months of rearing, indicated that fish reared in both seminatural treatment tanks consistently displayed a more olive-brown coloration, larger and darker parr marks, and darker spots than fish reared in the conventional treatment tanks. Fish in the conventional treatment tanks always appeared light tan and had poorly developed parr marks and few noticeable spots. Even after fish were removed from the tanks and anesthetized in MS-222, these differences in background skin coloration persisted. On average, the parr marks of seminaturally reared fish occupied a greater percentage of body surface than the parr marks of conventionally reared fish (Table 5-3). This percentage appeared to increase in proportion to the grain size used in the rearing environments. Fish reared over course-grained gravel had the largest parr marks, and those reared over fine-grained sand had the next largest parr marks, although their parr marks were similar in size to those of fish reared in extremely fine-grained conventional tanks.

In essence, fish from all three treatments developed cryptic skin coloration that blended with the background they were reared over: Conventionally reared fish developed a homogenous bright grey coloration that enabled them to blend in with the light uniform grey background coloration of their tank. Similarly, fish reared in the seminatural treatment tanks developed the dark, mottled, tan background coloration of their rearing environment. It is generally recognized that the former color pattern is cryptic in the open water environment of lakes and the ocean, while the latter pattern is cryptic in the more structurally complex environments of streams, rivers, and estuaries.

Thus, lighter colored conventionally reared fish were cryptically mismatched to the stream bed background of Anderson Creek, while seminaturally reared fish should have been cryptic in that environment. Fiih can quickly alter melanophore dispersion to make existing parr mark and spot patterns match environmental background. However, other facets of camouflage patterning, such as changes in hue, which require new pigment synthesis, take weeks to complete (Fuji 1993). Until all aspects of background matching were fully developed, the conventionally reared fish in this study should have remained more conspicuous in the stream.

Post release Survival

A significantly greater proportion of fall chinook salmon recovered at the weir were reared under seminatural than conventional conditions (60.1 vs. 39.8%, P = 0.007) (Table 5-4). Most fish were recovered at the weir within 3 days after their release into Anderson Creek As there were no weir failures, and no chinook salmon were captured when the creek was electrofished, recovery presumably represents survival. Predation may have been responsible for the majority of

Table 5-2. Base skin colorimetry values for fall chinook salmon reared in barren, gravel, and sand habitats, 1992.

	Habitat type				
	Unstructured				Post Hoc
Variable	Barren	Gravel	Sand	P value[a]	grouping[b]
Brightness				0.942	B S G
n	36	37	39		
Mean	21.572	21.730	21.710		
SD	1.983	2.169	2.195		
Hue				0.000	B S G
n	36	37	39		
Mean	0.35 1	0.364	0.362		
SD	0.011	0.011	0.013		
Chroma				0.000	B S G
n	36	37	39		
Mean	0.377	0.396	0.392		
SD	0.017	0.016	0.017		

[a] Probability of difference between treatments; values are based on ANOVA.

[b] Grouping of statistically similar and different treatments; determined by Tukey Test.

Table 5-3. Parr mark characteristics of fall chinook salmon reared in barren, gravel, and sand habitats, 1992.

	Habitat type				
	Unstructured	Structured			
Variable	Barren	Gravel	Sand	P value[a]	Post Hoc grouping[b]
Parr mark (% body area)				0.018	B S G
n					
Mean	25.6	346.3	30 5.8		
sd	1.0	1.0	0.9		
Dorsal spot count				0.758	G B S
n					
Mean	27 10.11	29 9.552	35 10.086		
sd	3.826	2.720	3.166		

*Probability of difference between treatments; values are based on ANOVA.

[b] Grouping of statistically similar and different treatments; determined by Tukey Test.

Table 5-4. Number of Big Beef Creek fall chinook salmon released from each treatment into Anderson Creek and recovered at the estuary weir by 8 June 1992.

	Habitat type		
	Unstructured	structured	
Variable	Barren	Gravel	Sand
Number released	88	101	102
Number recovered	33	63	59
Number not recovered	50	38	43
Survival to weir (%)	39.8	62.4	57.8

postrelease mortality as 1) outmigration was rapid, 2) no chinook salmon appeared to take up residence within the creek, and 3) no fish were found dead or moribund at the weir.

Healey (199 1) indicated that predation is a major source of mortality for chinook salmon. Based on our personal observation of a single heron that fed on over 80 similar-sized trout within a few hours, and on information from the literature (Elson 1962, Wood 1987). it appears that avian predators are the greatest threat to newly released hatchery fish; however, losses to predatory fish and reptiles may also be significant. The main piscivorous predators observed in the vicinity of Anderson Creek were great blue herons, kingfishers, mergansers, garter snakes, sculpins, cutthroat trout, and rainbow trout. All of these animals are primarily visually hunting predators.

There are three main antipredator strategies available to an animal: 1) avoid areas where predators are found, 2) escape predators when attacked, and 3) be cryptic to avoid detection by predators. There is no reason to believe fish from any rearing treatment would be better able to avoid areas where predators were found. Within a healthy monospecific group, size has been shown to be the most important factor in escaping predators, once a fish has been detected The similarity in size of fish from each treatment suggests their ability to flee from predators would be equal. However, as noted above, the distinctive heterogeneous coloration of seminaturally reared fish probably enhanced their crypticity for the stream bed coloration of Anderson Creek more than the coloration of their homogeneously colored conventionally reared counterparts. Thus, coloration was the strategy that reduced predator vulnerability for fish from the seminaturally reared treatments.

Conclusions

Historically, salmonids have been reared in earthen raceways, similar to the seminatural raceways in this study. Piper et al. (1982) stated that many fish culturists believed that fish produced from these earthen raceways were healthier, more colorful, had better fin condition than those produced by concrete raceways. Our study results supported this view, and in addition, our seminaturally reared fish had better postrelease survival than fish grown in conventional, concrete-bottomed vessels. As we have pointed out, the primary advantage of providing seminatural habitats for rearing hatchery fish appears to be that fish reared under these conditions develop body coloration that is cryptic in postrelease stream environments.

This cryptic background color matching is a crucial component of the camouflage that enables prey to avoid detection by visually hunting predators. It appears that fish reared over naturally colored sand and gravel substrates in this study were less vulnerable to visually hunting predators (birds and fish) than conventionally reared fish. The mechanism promoting increased survival of the seminaturally reared fish appears to be enhanced crypsis in the stream environment.

Although this study does not examine whether seminaturally reared salmonids are exposed to the same selection pressures or exhibit the same behavior as wild fish, it does demonstrate that modification of the culture environment can induce significant positive differences in coloration and postrelease survival of hatchery fish. This is an important first step in developing seminatural culture habitats that can produce wild-like fish for use in genetic conservation and supplementation programs.

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Section 6

THE POSTRELEASE SURVIVAL OF YAKIMA RIVER SPRING CHINOOK SALMON ACCLIMATED IN CONVENTIONAL AND SEMINATURAL RACEWAYS, 19944

by

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Contents

Introduction

This experiment was a continuation of research begun in 1992. This study was conducted in 1994 and compared the postrelease survival of spring chinook salmon (*Oncorhynchus tshawytscha*) reared with substrate, instream structure, and overhead cover to survival of conventionally reared fish. The research was conducted with spring chinook salmon to determine if their response to seminatural rearing is similar to that described for fall chinook salmon in Section 5. In this experiment, fish were exposed to seminatural rearing for only a few months prior to smolting.

Material and Methods

The research was conducted at the National Marine Fisheries Service (NMFS) Freshwater Fish Culture Laboratory at the University of Washington's Big Beef Creek Research Station near Seabeck, Washington. Test fish were 1992 brood-year spring chinook salmon reared from adults sourced from the Yakima River. Eggs were incubated and fish reared for 3 months in 2-m diameter circular tanks under the standard culture practices described by Leitritz and Lewis (1980) and Piper et al. (1982). In the fall of 1993, they were coded wire-tagged and adipose clipped so that when released in the Yakima River they could be identified from wild-reared fish.

Test rearing was conducted in rectangular 400-L acrylic tanks modeled after hatchery raceways. Each tank was 46 cm wide, 46 cm deep, 152 cm long and maintained at a water depth of 43 cm. Four liters per minute of 10°C well water was supplied to each tank through a 10-cm diameter opening. All tanks were supplied with air via four airstones spread along the bottom rear of each tank. Sheets of grey-black painted polystyrene were fitted to the outside of both ends, the rear, and the bottom of all tanks to simulate the grey concrete background coloration of a standard raceway. The experimental rearing vessels were set up outside, and the area between the banks was enclosed in a tent-like structure. The tanks were lit from the surface by ambient sunlight.

Experimental rearing vessels were identical to those used in the 1992 trials, with the exception of a grey curtain, which was added to enclose side walls to prevent fish from viewing adjacent tanks. In addition the facility was expanded with a second bank of 12 tanks to increase the number of replicates to 12 per treatment. The 12 conventional treatment tanks represented a standard raceway environment lacking any substrate, structure,. or overhead cover. The 12 seminatural culture treatment tanks all had ornamental junipers (Juniperous *horizontalis*) for instream structure, pea gravel over undergravel filters for substrate, and black opaque aquarium covers for overhead cover. The undergravel filters in the seminatural rearing treatment tanks were air driven. An equivalent volume of air was supplied to the conventional tanks.

Experimental rearing began on 25 January 1994. Tanks were stocked with 80 spring chinook salmon each over a 3-day period. Stocking involved netting fish from a common population, anesthetizing them in MS-222; measuring fork length to the nearest mm, weighing fish to the nearest gram, and then randomly assigning them to the 24 tanks.

During the experiment, fish were generally maintained following standard hatchery practices. The fish in each tank were hand fed an equal weight of a commercially prepared pellet diet daily from the surface. Every week, the tanks were cleaned with an aquarium siphon vacuum to remove algae, fungus, feces, and decaying food particles. All mortalities were removed and counted daily. Unlike the previous fall chinook salmon experiment, no data were collected on fish behavior during the trial period.

From 6 to 7 April 1994, all study fish were again anesthetized in MS-222, their fork lengths were measured to the nearest mm, and fish were weighed to the nearest gram. Every twentieth fish was sacrificed in a lethal concentration of MS-222. Each sacrificed fish was then submerged on its side in a shallow tray filled with water and photographed for skin color and morphometric analysis under standardized lighting conditions.

Euthanatized fish were dissected and examined for the presence of bacterial disease organisms in the kidney. A sterile inoculation loop was dipped into the kidney tissue and streaked across a prepared media in a petri dish. Petri dishes were incubated at room temperature, and after 24 hours they were examined for the presence of furunculosis (Aeromonas salmonicida) or enteric redmouth (Yersinia *ruckeri*) organisms. After plating, the kidney was removed and homogenized and a clean cotton swab was used to streak homogenized kidney across glass slides. Prepared slides were examined for the presence of Renibacterium *salmoninarum*, the causative agent of bacterial kidney disease (BKD), using the florescent antibody technique (FAT) described by Bullock and Stockey (1975).

Remaining fish were then tagged with passive integrated transponders (PIT) tags, following the method described by Prentice et al. (1990). Fish were then returned to their tanks and held until released for postrelease survival evaluations.

Fish were released back to their native Yakima River in two paired releases (on 15 April and 19 April 1994). In each release, fish from six conventional and six seminatural treatment tanks were loaded into insulated 2,000-L fish transport tanks supplied with continuously oxygenated water. Fish were then immediately transported to the upper Yakima River (about a 4-hour trip) where they were released midday at river km 314 and challenged to survive a 225-km outmigration, past the juvenile fish collection facility at Prosser Dam on the lower Yakima River, and onto the juvenile fish collection facility at McNary Dam on the Columbia River for an overall migration of 370 km.

Proportions of fish detected at Prosser Dam and McNary Dam were compared. Recovery proportions were actual numbers of fish recorded by PIT-tag interrogation systems on fish collection facilities at the dams, and were not expanded to include fish that bypassed the dams by other (e.g., spillway or turbine) routes. Expansion of data was not considered necessary for relative comparisons between release groups. However, recovery proportion data can typically be expanded by 50% or more to estimate all fish passing (Ruehle and McCutcheon 1994). Therefore, recovery data reported in this study should be viewed as a minimal estimate of survival between groups.

Differences in recovery between treatments were statistically analyzed with a 2 by 2 contingency table. The migration rate of fish was based on the time between release and detection at the Prosser Dam PIT-tag interrogation facilities. Travel time from release to Prosser Dam was analyzed with a fully factorial ANOVA.

Results and Discussion

Growth and Survival During Rearing

Survival of fish from both treatments was high, exceeding 99% during rearing (Table 6-l). Tagging mortality was also low, not exceeding 0.2% in either group. During the study, seminatural rearing of spring chinook salmon had no statistically detectable positive or negative effect on the in-culture ($P = 0.547$) or tagging ($P = 0.50$) survival of spring chinook salmon , compared to conventional rearing (Table 6-l). This result was similar to our previous findings (Section 5), which indicated that seminatural rearing techniques did not compromise survival of fall chinook salmon. The overall excellent fish health in both studies may be attributable to rearing fish in disease-free well water.

There was no significant difference ($P < 0.68$) between treatments in the fork length of fish at the beginning of the experiment, and this suggested randomization of fish for the experiment was successful (Table 6-2). However, the conventionally reared fish were significantly ($P = 0.000$) longer and heavier than seminaturally reared fish by the time of PIT tagging. Both groups were fed an identical ration, so the difference in growth had to be attributed to a difference between treatments. In the seminatural tanks, we observed that feed often became unavailable to the fish when it lodged in the gravel and plants. We believe this, and not some other aspect of seminatural tearing, was responsible for the difference in growth between fish in the two treatments. Sacrificed fish from both treatments had a low incidence of BKD (Table 6-3) as measured by FAT. None of the bacterial culture plates were positive for *Aeromonas* sp., Pseudomonas sp., or Yersinia *ruckeri*, although a few plates were positive for unidentified microorganisms. A 2 by 2 contingency table analysis revealed no significant difference in BKD incidence ($P = 0.7\ 14$) or microbial culture growth ($P = 0.217$) between the two treatments.

Raceway Maintenance

In this study, as in the study described in Section 5, the 400-L rearing containers fouled more quickly than other raceway systems we have worked with. However, the substitution of junipers for instream structure reduced the growth of filamentous algae from the amounts found when using plastic aquaria plants in the previous study (Section 5). Maintenance of each 400-L seminatural rearing vessel required about 0.4 hours/week, while maintenance of each 400-L conventional rearing vessel required about 0.2 h/week.

Morphology

The photograph quality of the subsamples was too poor for body-color analysis using current technology. However, our subjective observations indicated that fish reared in seminatural treatment tanks consistently displayed a more olive-brown coloration, larger and darker parr marks, and darker spots than fish reared in the conventional treatment tanks. These background skin coloration differences persisted even after fish were removed from the tanks and anesthetized in MS-222. These differences were similar to those observed with fall chinook salmon in our 1991-1992 study (Section 5). Both studies supported the assumption that seminatural rearing promotes the development of cryptic coloration for the postrelease environment.

Table 6-1. In-culture survival of Yakima River spring chinook salmon reared in seminatural and conventional treatments, 1994.

Variable	First Release		Second Release	
	Seminatural	Conventional	Seminatural	Conventional
Number of:				
Rearing vessels	6	6	6	6
Fish ponded	480	480	480	480
Known mortalities				
Siphon (cleaning)	0	0	2	0
Jumpers	0	0	0	1
Unexplained mortalities	4	2	3	3
Lethal samples	24	24	24	24
Fish tagged[a]	452	454	453	453
Post tagging mortalities	0	2	0	0
Rejected tags[b]	14	9	5	2
Tagged fish released[c]	438	443	448	451
Rearing survival (%)	99.1	99.1	99.4	99.4

[a] Probability of difference between seminatural and conventional survival: combined releases, P = 0.547; values are based on student t-test.

[b] All fish that rejected PIT tags survived but were not used for release evaluation.

[c] Probability of difference between seminatural and conventional survival: combined releases, P = 0.50; values are based on student t-test.

Table 6-2. Length and weight of Yakima River spring chinook salmon reared in seminatural and conventional treatments, 1994.

Variable	First Release		Second Release	
	Seminatural	Conventional	Seminatural	Conventional
Size at Ponding:				
Length (mm)[a]				
n	445	446	480	480
mean	110.2	110.3	110.8	110.6
sd	6.3	6.4	6.2	7.1
Size at Tagging:				
Length (mm)[b]				
n	476	479	477	477
mean	130.9	133.0	131.7	133.8
sd	6.2	6.9	6.2	7.0
Weight (g)[c]				
n	476	459	477	477
mean	23.6	24.9	23.1	24.5
sd	4.3	4.8	3.8	4.6

[a] Probability of difference between seminatural and conventional: release 1, P = 0.773; release 2, P = 0.684; values are based on student t-test.

[b] Probability of difference between seminatural and conventional: release 1, P = 0.000; release 2, P = 0.000; combined, P = 0.000; values are based on student t-test.

[c] Probability of difference between seminatural and conventional: release 1, P = 0.000; release 2, P = 0.000; combined, P = 0.000; values are based on student t-test.

Table 6-3. **Health status** of Yakima River spring chinook salmon reared in seminahiral and conventional treatments, 1994.

Variable	First Release		Second Release	
	Seminatural	Conventional.	Seminatural	Conventional
BKD[a]				
n	24	24	24	24
No. Positive	0	0	0	0
No. Negative	24	24	24	24
Infection Rate (%)	0	0	0	0
Fields Positive (Infected Only)	0	0	0	0
Mean No. BKD/Field (Infected Only)				
mean	0	0	0	0
sd	0	0	0	0
se	0	0	0	0
Culture Plates[b]				
Growth	2	4	1	1
No growth	22	20	23	23

[a] Probability of difference between seminatural and conventional: combined releases, P = 0.7 14; values are based on 2 x 2 contingency analysis.

[b] Probability of difference between seminatural and conventional: combined releases, P = 0.217; values are based on 2 x 2 contingency analysis.

Postrelease Survival

The first release of fish to the Yakima River occurred on 15 April 1994, with fish liberated into clear water with an estimated secchi disk visibility greater than 3 m. Washington Department of Fish and Wildlife (WDFW) personnel snorkeling at the site observed the fish as they were released into the river. The second paired release of fish from the remaining 12 tanks occurred on 19 April 1994, when water at the release site was extremely turbid with an estimated secchi disk reading of less than 15 cm. WDFW personnel did not'snorkel to observe fish on the second release because of this poor visibility.

Most fish reached the Prosser Dam PIT-tag interrogation facilities within 2 weeks of their release into the upper Yakima River (Table 6-4). For the clear-water release, mean travel time to Prosser Dam was 11.4 days for conventional treatment fish and 11.6 days for seminatural treatment fish. Mean travel time was faster for both treatment groups under turbid water conditions (conventional = 9.5 days, seminatural = 10.5 days). Factorial ANOVA indicated no significant difference (P c 0.382) in travel time between treatments for either release. However, fish in the second release reached Prosser Dam significantly (P = 0.021) sooner (1.5 days) on average than fish in the first release. The slightly higher water velocity, caused by flood run-off on the second release, can probably account for some (at most 6 hours) of this increased speed. However, the poor visibility in the second release may also have been a factor in increased travel time: the turbid conditions may have prevented fish from holding position or may have stimulated migration behavior.

Significantly more fish from combined treatments were recovered at Prosser Dam from the second than the first release (32.0 vs. 24.54, $P < 0.001$). This may have been related to water clarity, travel speed, or increased capture efficiency at the interrogation sites. We believe the first hypothesis is probably correct, as all fish would be much less visible to visually hunting predators in murky water.

An almost significantly greater proportion of the seminaturally reared fish than conventionally reared fish (27.2 vs. 21.9%, $P = 0.072$) were recovered at Prosser Dam from the clear-water releases (Table 6-4). This resulted in a 24% increase in relative survival of seminaturally reared fish over conventionally reared fish. This increase may be attributed to the enhanced crypsis of the seminatural-reared fish when viewed against the heterogenous riverine background observed in the clear-water release.

In the second release, made under turbid water conditions that created a homogenous riverine background, there was a slight but nonsignificantly greater recovery of fish at Prosser Dam from the conventional than seminatural rearing environment (33.7% vs. 30.6%). The darker heterogenous integument of seminaturally reared fish should have contrasted with the light homogenous background generated by turbid water. In contrast, the lighter homogenous coloration of conventionally reared fish should have blended in with the background. Thus, seminaturally reared fish theoretically should have been more conspicuous to visually hunting predators in this turbid water environment. This leads us to conclude that water clarity must be considered before release of seminaturally reared fish is implemented.

Table 6-4. Postrelease recovery and travel time for Yakima River spring chinook salmon reared in seminatural and conventional treatments and released in the Yakima River, 1994.

Variable	First Release		Second Release	
	Seminatural	Conventional	Seminatural	Conventional
Number of:				
Tagged fish released	438	443	448	451
Fish reaching:				
Prosser Dam[b]	119	97	136	152
McNary Dam	76	56	80	92
Recovery (%):				
Prosser Dam[c]	27.2	21.9	30.6	33.7
McNary Dam	17.4	12.6	17.9	20.4
Travel time to:				
Prosser Dam[d]				
n fish analyzed	83	59	96	106
mean days	11.6	11.4	10.5	9.5
sd	5.5	6.1	5.9	6.2
McNary Dame				
n fish analyzed	76	56	80	92
mean days	20.1	18.4	17.9	18.0
sd	5.2	4.7	5.4	6.2

[a] Fish in first release released into clear water river conditions; Fish in second release released into turbid water river conditions.

[b] Prosser Dam recoveries include fish detected at McNary Dam that were not detected at Prosser Dam.

[c] Probability of difference between seminatural and conventional recovery: Release 1, P = 0.072, Release 2, P = 0.285; values are based on 2 x 2 contingency analysis.

[d] Probability of difference between seminatural and conventional travel time: Release P = 0.021, Treatment P = 0.382, Interaction P = 0.564, values are based on factorial ANOVA.

[e] Probability of difference between seminatural and conventional travel time: Release P = 0.049, Treatment P = 0.186, Interaction P = 0.152; values are based on factorial ANOVA.

Conclusions

It appears that the postrelease benefits of seminatural rearing for fall chinook salmon described in Section 5 can be extended to spring chinook salmon. In addition, it appears that administration of seminatural rearing protocols at the end of the rearing cycle just prior to release will increase postrelease survival of hatchery fish. However, longer rearing in seminatural conditions may provide benefits to spring chinook salmon beyond those observed in this study.

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Section 7

THE POSTRELEASE SURVIVAL OF SATSOP RIVER FALL CHINOOK SALMON REARED IN CONVENTIONAL AND SEMINATURAL RACEWAY HABITATS, 19945

by

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Contents

Introduction

The goal of this final experiment was to test on a larger scale the seminatural rearing methods that had been shown to be effective in pilot-scale experiments (Sections 5-6). This experiment was conducted in 1994 as a cooperative project between the National Marine Fisheries Service (NMFS) and the Washington Department of Fish and Wildlife (WDFW). The study was conducted with fall chinook salmon (*Oncorhynchus tshawytscha*) reared in 6,000-L vessels (400-L vessels were used in our previous studies).

Material and Methods

This experiment was conducted at the Washington Department of Fish and Wildlife (WDFW) Simpson Fish Hatchery in the Satsop River Basin in western Washington. Experimental fish rearing was conducted in six rectangular 5,947-L portable fiberglass raceways. The concrete, grey-colored raceways were 6.4 m long by 1.5 m wide by 0.6 m deep.

The three conventional treatment raceways represented a conventional culture environment (Leitritz and Lewis 1980, Piper et al. 1982), lacking any substrate, structure, or overhead cover. However, they were covered with translucent bird netting on PVC pipe frame to prevent entry of avian predators. Conventional treatment fish were fed a standard pellet diet at the surface by hand.

The three seminatural treatment raceways (Fig. 7-l) were semi-production-level versions of the 400-L tanks used in the previous studies described in Sections 5 and 6. Overhead cover, which simulated stream-bank vegetation, was provided in each seminatural raceway by running military camouflage netting along the top of each side so that 80% of the tank surface was covered. The open area down the center of the raceway was covered with bird netting. This cover configuration simulated the canopy produced by riparian vegetation along streams.

The bottom of each seminatural raceway was covered with a 10-cm layer of pea gravel over undergravel filters constructed from perforated aluminum plate on a 5- by 5-cm aluminum box frame. Instream structure was created by placing five heavily branched, small (1- 1.5 m) sheared Douglas fir (*Pseudotsuga* menziesii) trees in each seminatural raceway. The needles were removed from all trees before they were added to the raceways. An automatic vibrating-type hopper feeder dispersed a standard pellet diet into a water current traveling through a 2.5-cm diameter pipe that encircled the perimeter of the raceway (Fig. 7-2). The feed-delivery pipe was laid over the substrate and delivered food through thirteen 7-cm-diameter holes drilled at 0.5-m intervals in the topside of the pipe.

In fall 1993, chinook salmon eggs were randomly divided among five Heath-type incubator trays and thermally marked, following the method of Volk et al. (1990). Eggs placed in these 5 trays were all from a pooled population taken from 5-10 females on a single day. A sixth tray of fish was established from a group of eggs taken from another pooled population of 5- 10 females spawned the following day. At swimup on 21 March 1994, the fry were individually counted, and each experimental raceway was stocked with 6,000 fry.

Fish in both treatments were maintained following standard culture practices (Leitritz and Lewis 1980, Piper et al. 1982). Throughout most of the rearing period, fish from both treatments were fed equivalent rations of commercially prepared dry and semi-moist diets. All mortalities during culture were removed and counted. Fish that died by jumping from the tanks or by injuries from cleaning siphons were counted separately from fish dying from undetermined causes.

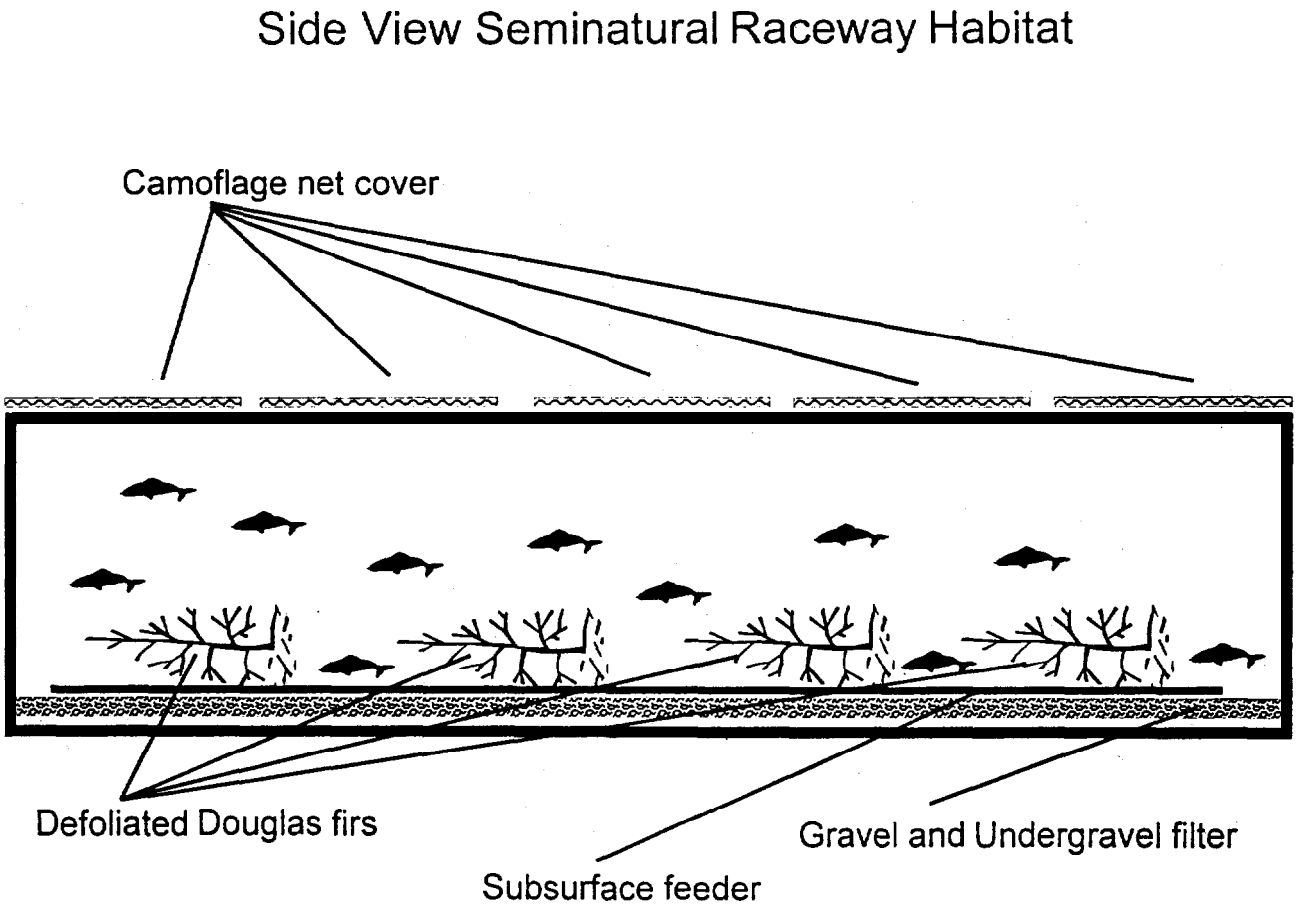
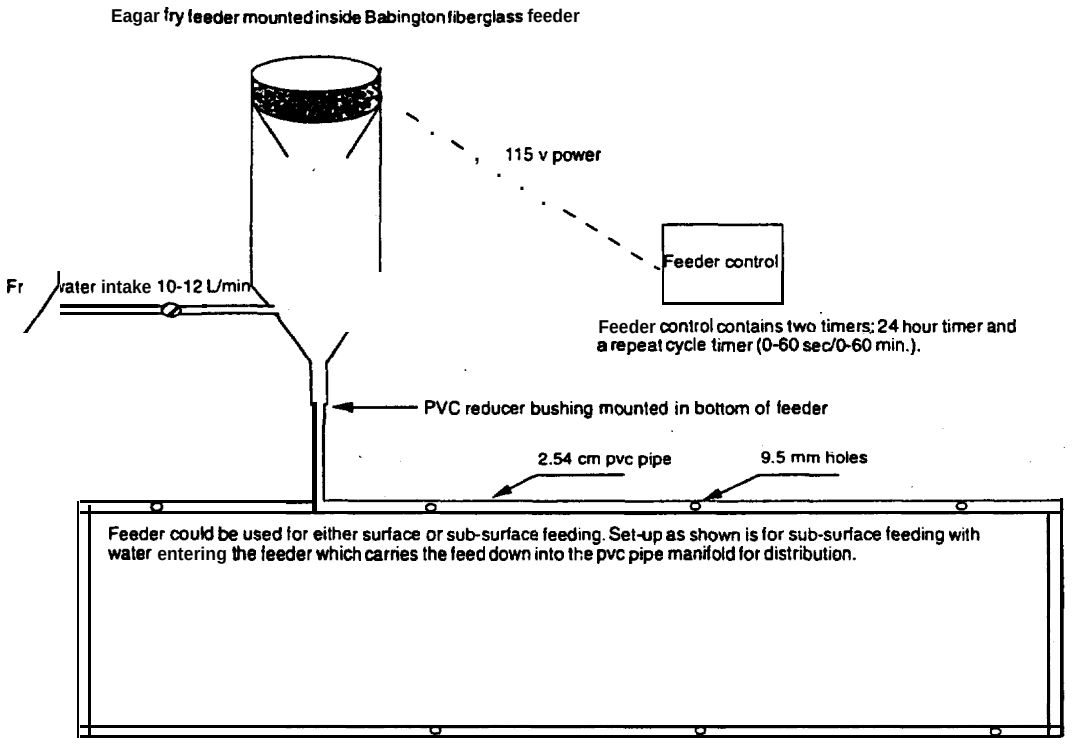
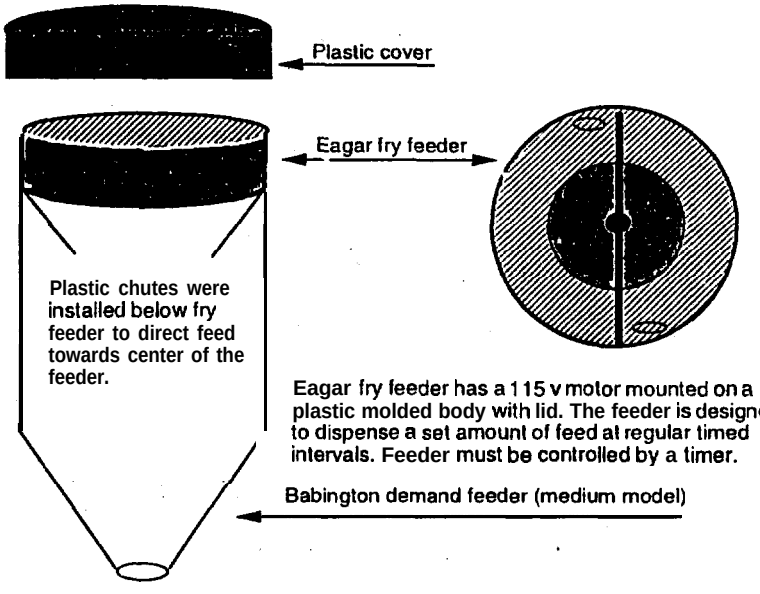


Figure 7-1. Seminatural raceway habitat that Satsop River fall chinook salmon were reared in at WDFW Simpson Hatchery.



Automatic feed system utilizing a Babington fiberglass feeder with a lop-mounted Eagar fry feeder.



Fry feeder has a 1.2 cm lip around the top perimeter of the feeder. Clearance between the inside wall of the fiberglass feeder and the outside wall of the fry feeder is less than 6 mm allowing the fry feeder to be inserted into the top of the fiberglass feeder. The lip of the fry feeder secures it inside and on top of the fiberglass feeder.

Feeder will hold a maximum 3 kg.

Figure 7-2. Subsurface feeding systems incorporated in seminatural raceway habitat that Satsop River fall chinook salmon were reared in at WDFW Simpson Hatchery.

Ten to 50 fish were anesthetized in MS-222 and sampled for growth (length) at the beginning of the experiment and at days 33 and 59. Just prior to release, a representative subsarnple of approximately 530 fish was taken from each raceway. Most of these fish were anesthetized in MS-222, measured for fork length, and PIT tagged following the methods of Prentice et al. (1990). Every twelfth fish in this sample was euthanatized in a lethal concentration of MS-222, measured, and photographed for cryptic coloration analysis. Each fish in the lethal subsample was submerged on its side in a shallow tray filled with water and photographed under standardized lighting conditions. The resulting photographic slides were then viewed on a video monitor, and the image was analyzed visually. The base skin color immediately below the dorsal fin and above the lateral line was matched up with a color chip according to the methods of Maerz and Paul (1950). The brightness, chrome, and hue of each color chip was then determined with a colorimeter. In addition, gill covers of photographed fish were examined for erosion.

The euthanatized fish were then dissected and examined for the presence of bacterial disease organisms in the kidney. A sterile inoculation loop was dipped into the kidney and streaked across a prepared media in a petri dish. Petri dishes were incubated at room temperature for 24 hours and then examined for the presence of furunculosis (Aeromonas *salmonicida*) or enteric redmouth (Yersinia *ruckeri*) organisms. After plating, the kidney was removed and homogenized. A clean cotton swab was then used to streak homogenized kidney tissue across glass slides, which were then examined for the presence of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), using the florescent antibody technique (FAT) described by Bullock and Stockey (1975).

During the last prerelease sampling period, an additional 500 fish were elastomer tagged for a WDFW comparative tagging study. Also, approximately 2,500 additional fish excess to experimental needs were removed from each raceway and liberated into the Satsop River. The remainder of fish, including both PIT- and elastomer-tagged fish, were returned to their raceways and held until release in postrelease survival studies.

Beginning in June 1994, fish were released to Bingham Creek in three paired groups (each including fish from a conventional and seminatural raceway) at l-week intervals. All groups were challenged to survive outmigration to a WDFW weir approximately 21 km downstream. The first release was made on 13 June 1994, during a period of rainfall. The second release was made on 20 June 1994, during a period with no rainfall and stable creek conditions. The third paired release was made on 27 June 1994, with a period of rainfall following several days later.

On each release day, fish from a pair of raceways were crowded, netted, and loaded for transport into an insulated 2,000-L tank with oxygenated water. Five hundred fish from each raceway were retained for in-culture behavioral observation and other studies. The fish in each release were liberated into Bingham Creek just before dusk (at approximately 2200 h).

Bingham Creek is a medium-size coastal stream with both logged and unlogged riparian habitat. Spawner access to the stream is controlled such that the stream supports a population of cutthroat trout (*O. clarki*), rainbow trout (0. *mykiss*), and coho salmon (0. *kisutch*), but lacks a chinook salmon run. Juvenile salmonid predators observed in the stream during the challenge included river otter (*Lutra canadensis*), great blue heron (*Ardea* herodius), belted kingfishers (*Ceryle alcyon*), and steelhead trout (0. *mykiss*).

The chinook salmon smolt outmigration was monitored at the trap for more than 90 days. The trap was designed to sample 100% of the downstream migrants. Just prior to end of weir operations, several sections of the creek were electrofished for residual study fish.

A PIT-tag detector system was used to interrogate all fish for their PIT-tag code as they entered the trap at the weir. This PIT-tag information provided an accurate measure of travel time to the weir. The reading efficiency of the PIT-tag detector installed at the Bingham Creek weir averaged about 82%. All fish entering the trap were manually sampled at the weir at 0700 h, 1500 h, and 2100 h for fork length (mm) and PIT-tag code.

Results and Discussion

Growth and Survival During Rearing

Total in-culture survival was high; mortality did not exceed 1.33% for either treatment prior to tagging (Table 7-l). Mortality due to known mechanical damage, jumping out of raceways, and sampling were similar for both rearing treatments (Table 7-1). However, mortality from other undefined causes was significantly ($P < 0.001$) higher for fish from seminatural than conventional raceways (about 1.2 vs. 0.6%). This increased mortality for seminaturally reared fish may have. arisen from disease or unobserved mechanical damage that occurred during cleaning.

When ponded, conventionally and seminaturally reared fish did not significantly differ in size (Table 7-2). However, by day 59 the conventionally reared fish were significantly longer ($P = 0.051$) and heavier ($P = 0.007$) than the seminaturally reared fish (Table 7-2). The subsurface feeders on the seminatural raceways failed to deliver an estimated 10% of daily ration. We estimate that the initial growth advantage for conventional fish approximately matched what the subsurface feeder failed to deliver. This suggested that if fish from the seminatural treatment had been presented with an equivalent food ration, their growth would have been similar.

Conventional fish were taken off feed for several days to allow seminaturally reared fish to attain equal size. At release, seminaturally reared fish were similar in length and only slightly lower in weight than conventionally reared fish (Table 7-2).

As in the previous two studies (Sections 5 and 6), no differences in health status were observed between fish from the conventional and seminatural rearing treatments. Bacterial colonies produced from kidney streaks on agar culture plates occurred in nearly equal proportions from fish subsampled from both treatments ($P = 0.7 12$) (Table 7-3). Similarly, only slightly more fish from the conventional raceways were positive for BKD, and this difference was not significant ($P = 0.217$).

In photographs of the lethal subsamples, only 2% of conventionally reared fish had eroded gill covers, while 22.9% of those of the seminaturally reared fish were eroded. A 2 x 2 contingency table analysis indicated difference was highly significant ($P < 0.001$)~ The actual cause of gill cover damage in unknown. However, if disease-related, then prolonged rearing under seminatural conditions may affect survival.

Raceway Maintenance

Both conventional and seminatural raceways required weekly siphoning to vacuum fungus, fecal material, decaying food, and sediment from the tank bottoms. Structure had to be removed from the seminatural raceways to allow thorough cleaning. When the conifers were removed during the cleaning process, an effort was made not to disturb the epiphyte growth on them Complete cleaning required about 1 hour for each conventional raceway and 2 hours for each seminatural raceway. Gravel beds above the undergravel filters also collected sediment and usually

Table 7-l. Number, percentage, and cause of fall chinook salmon mortalities in conventional and seminatural raceways during rearing at WDFW Simpson Hatchery, 1994.

Source	Conventional	Seminatural
Mechanicaldamage	10 (0.06%)	9 (0.05%)
Jumpers	19 (0.11%)	15 (0.08%)
sampling	7 (0.04%)	6 (0.03%)
Unknown causes	101 (0.56%)	209 (1.16%)
TOTAL	137 (0.76%)	239 (1.33%)

Table 7-2. Fork length of fall chinook salmon reared in conventional and seminatural raceways at WDFW Simpson Hatchery, 1994.

	Conventional			Seminatural		
	Raceway			Raceway		
Response variable	1	2	3	1	2	3
Ponding						
n sampled	50	50	50	50	50	50
mean length (mm)	39.5	39.8	39.8	39.8	40.0	39.9
sd	1.3	1.0	1.1	1.2	1.3	1.0
Day 33						
n sampled	10	10	10	10	10	10
mean length (mm)	51.9	49.7	48.6	51.3	48.8	49.3
sd	3.0	3.0	3.0	3.0	3.0	3.0
Day 59						
n sampled	10	10	10	10	10	10
mean length (mm)	64.9	65.1	62.1	63.7	61.6	60.4
sd	4.0	4.0	4.0	4.0	4.0	4.0
Release*						
n sampled	108	98	104	106	100	106
mean length (mm)	70.4	76.0	81.3	69.7	76.1	80.3
sd	4.5	4.6	4.9	3.9	4.1	3.8

[a] Release dates were 13 June 1994 for paired conventional and seminatural raceways 1, 20 June 1994 for paired conventional and seminatural raceways 2, and 27 June 1994 for paired conventional and seminatural raceways 3.

Table 7-3. Health status at PIT tagging for fall chinook salmon reared in conventional and seminatural raceways at WDFW Simpson Hatchery, 1994.

Variable	Conventional	Seminatural	Probability[a]
Number of plates			
Negative	109	112	0.712
Target positive[b]	0	0	
Nontarget positive[c]	3	8	
Number of fish tested for BKD			
Negative	109	114	0.217
Positive	8	3	

[a] Probability of difference between treatments; values are based on student t-tests.

[b] Target organisms were Aeromonas sp., Pseudomonas sp., and Yersinia *ruckeri*.

[c] Nontarget organisms were colonies not attributable to *Aeromonas* sp., *Pseudomonas* sp., or Yersinia *ruckeri*.

required over twice as much time to clean as the conventional raceway bottoms. The sidewalls of conventional raceways required scrubbing to remove algal growth. In an effort to maintain a natural rearing environment, sidewalls of seminatural raceways were not scrubbed, reducing overall labor by about 30%.

As noted above, the subsurface feed system in the seminatural tanks often partially clogged and failed to deliver the total feed ration. In addition, this feed system required 5- 10 minutes to disassemble and flush once or more each week. Nevertheless, the automated feeding system reduced labor compared to hand feeding in the conventional treatment. In future experiments, the subsurface feeder will be modified with clean-out ports to the outside of the raceway. This will allow easy daily flushing to ensure delivery of total ration.

Fish Behavior

The behavior of fall chinook salmon reared in conventional versus seminatural raceways differed markedly. Fish in seminatural raceways were oriented lower in the water column than conventionally reared fish. This benthic-to-midwater orientation appeared to be a result of subsurface presentation of food by the automated feed system in the seminatural treatments. Conversely, the midwater-to-surface orientation of conventionally reared fish appeared to result from the surface presentation of food.

Fish in conventional raceways scrambled in their competition for food. The introduction of pellets at the surface in conventional raceways seemed to induce fish to rush to the surface, where they formed dense clusters.

In seminatural raceways, dominant fish defended feeding territories (despotic competition) around holes in the subsurface feeder. However, when pellets were introduced, subordinate fish formed feeding groups that overwhelmed the dominant fish. The habitat structure in seminatural raceways, coupled with the despotic competition for feeding sites resulted in seminaturally reared fish being more dispersed throughout the raceway than conventionally reared salmon.

Even when not fed, salmon in conventional raceways swarmed to the surface every 15 minutes or so. This swarming behavior was catalyzed by a single insect or dust particle landing on the water surface. When a similar object broke the surface in seminaturalraceways, only a single fish pursued it.

Salmon in seminatural raceways were more polarized (better aligned to one another) than those in conventional raceways. It is unknown whether the structure, cover, feeding methodology, or substrate in seminatural raceways induced salmon to form more polarized groups than in conventional raceways.

Preference for decreased water column depth can increase vulnerability to aerial piscivores (Kramer 1983, 1987). Theoretically, the benthic-to-midwater orientation of seminaturally reared fish should decrease their susceptibility to avian predation. In contrast, the surface-feeding behavior of conventionally reared fish should attract fish-eating birds.

Morphology

As in the previous studies (Sections 5 and 6), the body color of seminaturally reared fish was noticeably more vivid than that of conventionally reared fish. Results from a nested ANOVA of subsamples at tagging indicated that integument brightness, hue, and chroma were significantly

different for fish between raceways within treatments (P = 0.008, P = 0.003, P = 0.001, respectively, Table 7-4). However, significant differences between treatments could only be detected for integument brightness (P = 0.006).

Nevertheless, our subjective observations suggested that to the human eye the coloration of fish within treatments was similar, while that of fish from different treatments strongly contrasted. The integument color of seminaturally reared fish visually matched the brown substrate they were reared over, while that of conventional fish visually matched the light grey of the raceway bottom

In addition, the seminaturally reared fish appeared to have more extensive melanophore development in the caudal fin, anal fm, abdominal area, and gill cover margin than conventionally reared fish. The parr marks of seminaturally reared fish were visually more pronounced both in culture and during photography. In the first few days after release, personnel examining fish trapped at the weir felt they could distinguish conventional treatment from seminatural treatment fish based on their coloration. These color differences appeared to begin to diminish within a few days after release, with conventionally reared fish seeming to develop coloration similar to that of seminaturally reared fish.

Postrelease Survival

Average travel time for the fish to cover the 21 km distance from the release site to the weir ranged from 11.0 to 14.4 days for seminaturally reared fish and 13.9 to 19.3 days for the conventionally reared fish (Table 7-5). Average travel time was shortest for fish in the last release group and longest for fish in the second release group (Table 7-5). In a two-way ANOVA, the most important factor affecting travel time was release date ($P < 0.001$), not treatment type ($P = 0.102$). The interaction between treatment type and release date was marginally significant ($P = 0.054$), and there was no significant difference ($P = 0.102$) in travel time between treatments. These-findings were similarto those of the spring chinook salmon study (Section 6), where seminatural rearing with substrate, cover, and structure also did not have any apparent effect on migratory speed.

Travel rates of 15 to 30 km/day have often been observed for fish in Columbia River system streams (T. Flagg, NMFS, unpublished data). In the present study, travel rates ranged from 1.5 to 1.9 km/day for seminaturally reared fish and 1.1 to 1.5 km/day for the conventionally reared fish. Therefore, we believe that the average travel time observed in this study reflected the length of time it took fish to initiate migration rather than the time of active migration through the reach.

Fish in the last release group probably migrated most rapidly because they were undergoing smoltification and were released close to the stock's natural outmigration time period (July 1). In addition, rainfall occurred immediately after their release. Fish in the second release probably took longer to initiate their migration because they were released earlier than the natural outrnigration time period and were not stimulated to migrate due to a lack of rainfall during the week after their release. Even though they were the smallest, and were released at the date farthest from their natural migration time, the travel time of fish in the first release was intermediate to the other two, probably because of the heavy rainfall immediately after their release.

Table 7-4. Base skin colorimetry values at PIT tagging for fall chinook salmon reared in conventional and seminatural raceways at WDFW Simpson Hatchery, 1994.

	Conventional			Seminatural		
	Raceway			Raceway		
Response variable	1	2	3	1	2	3
Brightness[a]						
n sampled	40	29	37	28	41	30
mean	50.2	49.1	46.1	40.9	41.9	40.5
sd	1.5	1.5	5.2	6.9	6.1	6.8
Hue[b]						
n sampled	40	29 17	37	28	41	30
mean	14.9	..2	14.3	12.7	15.2	10.9
sd	3.1	2.3	5.8	6.4	6.4	6.1
Chroma[c]						
n sampled	40	29	37	28	41	30
mean	2.4	3.3	3.2	3.4	3.3	3.2
sd	2.1	1.9	1.9	1.7	2.1	1.9

[a] Treatment P = 0.006, Raceway within treatment P = 0.008; values are based on nested ANOVA.

[b] Treatment P = 0.193, Raceway within treatment P = 0.003; values are based on ANOVA.

[c] Treatment P = 0.238, Raceway within treatment P = 0.001; values are based on ANOVA.

Table 7-5. Average time (days) required for fall chinook salmon reared in conventional and seminatural raceways at WDFW Simpson Hatchery to migrate the 21 km from the release site to recapture weir on Bingharn Creek, 1994.

	Conventional[a]			Seminatural[a]		
	Raceway			Raceway		
Response variable	1	2	3	1	2	3
Travel time (days)[b]						
n sampled	171	129	86	187	168	177
mean days	17.8	19.3	13.9	14.4	21.8	11.0
sd	17.6	15.8	14.5	18.3	16.6	13.3

[a] Conventionally and seminaturally reared fish released in paired groups 21 km above Bingham Creek weir. Raceways pairs number 1 were released on 13 June 1994, raceways pairs number 2 were released on 20 June 1994, and raceways pairs number 3 were released on 27 June 1994.

[b] Release $P < 0.001$, Treatment $P = 0.102$, Interaction $P = 0.054$; values are based on ANOVA.

Overall, significantly more seminaturally than conventionally reared fall chinook salmon were recovered at the weir (48 vs. 38%, $P < 0.001$) (Table 7-6 and Figs. 7-3, 7-4, and 7-5). Thus, seminatural rearing appears to have increased relative recovery by 26% in this study. No chinook salmon were recovered by electrofishing in Bingham Creek, even though numerous juvenile trout and coho salmon were caught: this suggests that the chinook salmon had probably migrated from the stream reach. Thus, although this survey covered less than 2 km, the weir recovery data should be a reasonable estimate of postrelease survival differences that occurred between fish from the two treatments.

For the first and last releases, the difference observed in daily recovery of fish at the weir between treatments was greatest immediately after release and diminished with time (Figs. 7-3, and 7-5). As noted in Sections 2-3, conventionally reared fish may not begin to develop proper camouflage coloration for the stream environment until several days to weeks after release. Theoretically, conventionally reared fish should be less vulnerable to visual predators if they seek cover and hold position until they have developed proper cryptic coloration for their new environment. Therefore, the proportionally lower recovery of conventionally reared fish during the early postrelease recovery period for the first and third releases may have been due to greater vulnerability to predators during this transition period for cryptic coloration for the stream environment.

The daily recovery of fish from the second release was initially similar for both treatments, but began to diverge with time (Fig. 7-4). The protracted nature of recoveries from the second release was probably due to the fishes' incomplete smoltification and the low stream discharge immediately after release (as described above). We have no explanation why the recovery of seminatural vs. conventionally reared fish from the second release diverged with time (Fig. 7-4). However, other aspects of NATURES rearing (e.g., benthic orientation to structure) may have helped increase fish survival.

Conclusions

This research demonstrated that seminatural rearing techniques developed in pilot-scale studies (Sections 5-6 of this report) can be implemented in production fish rearing scenarios. These seminatural rearing techniques increase postrelease survival of fish in streams. As our previous studies demonstrated, the primary advantage of providing seminatural habitats for rearing hatchery fish appears to be that fish reared under these conditions develop body coloration that is cryptic in postrelease stream environments. This crypticity helps camouflage fish from visually hunting bird and fish predators and probably provides the strongest survival advantage derived from the seminatural rearing techniques we tested. However, it was apparent from the results of this study that automated underwater feeding systems can also reduce predator vulnerability by inducing benthic orientation in hatchery fish.

This research also demonstrated that modification of the culture environment can induce significant positive differences in behavior and postrelease survival of hatchery fish. This is an important step in developing seminatural culture habitats to produce wild-like hatchery fish for genetic conservation and supplementation programs. We believe the approaches described here and in other work (e.g., Thompson 1966, Olla and Davis 1989) can provide solutions for stock restoration programs seeking to produce fish with high survival rates that are similar to their naturally reared cohorts.

Table 7-6. Number of WDFW Simpson Hatchery fall chinook salmon released into Bingham Creek and recovered at the weir, 1994.

Conventional[a]			Semi-Natural		
	Raceway			Raceway	
Response variable	1	2	3	1	2	3
Number released	455	392	396	423	467	454
Number recovered	209	154	109	231	208	208
Number not recovered	246	238	287	192	259	246
Survival to weir (%)	45.9	39.3	27.5	54.6	44.5	45.8

[a] Conventionally and seminaturally reared fish released in paired groups 21 km above Bingham Creek weir. Raceways pairs number 1 were released on 13 June 1994, raceways pairs number 2 were released on June 20, 1994, and raceways pairs number 3 were released on 27 June 1994.

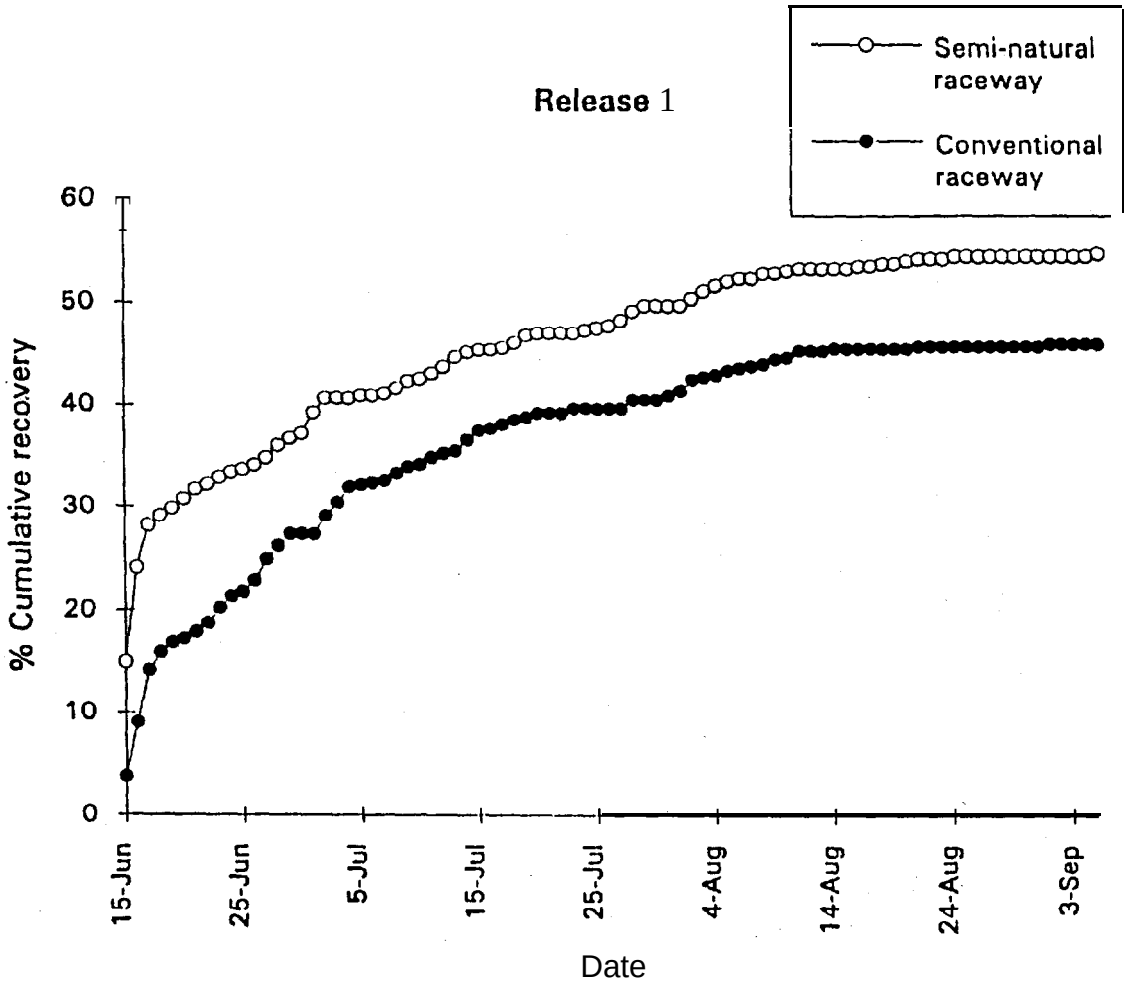


Figure 7-3. Cumulative recovery at Bingham Creek weir for conventionally and seminaturally reared fall chinook salmon from the **first** paired release, 1994.

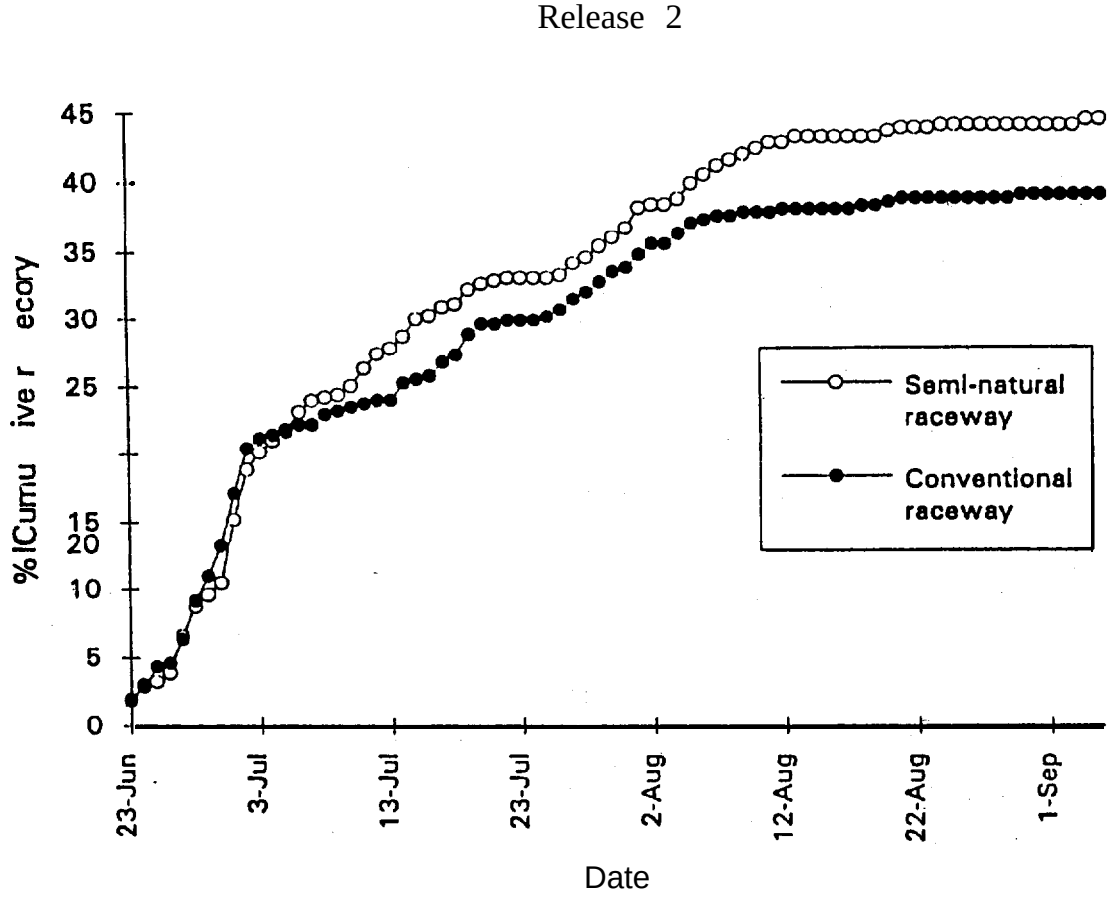


Figure 7-4. Cumulative recovery at Bingham Creek weir for conventionally and seminaturally reared fall chinook salmon from the second paired release, 1994.

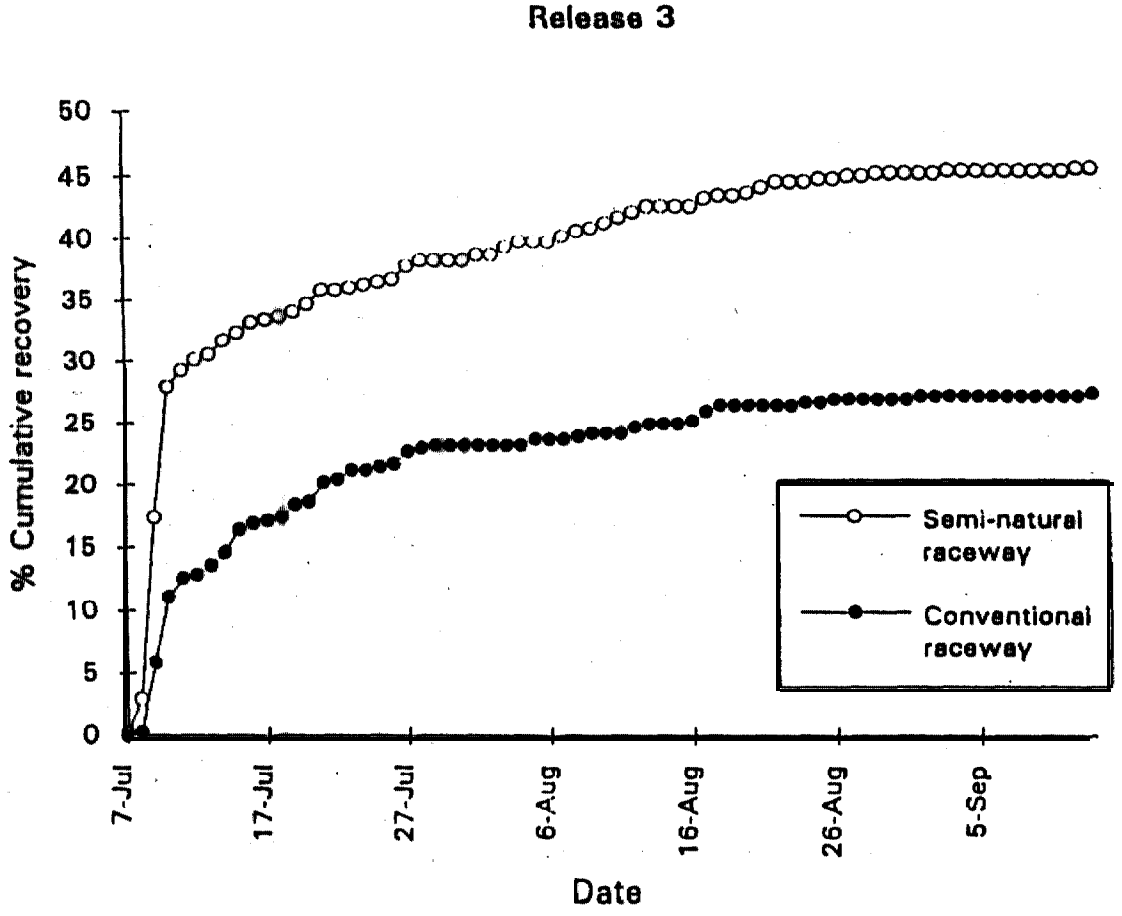


Figure 7-5. Cumulative recovery at Bingham Creek weir for conventionally and seminaturally reared fall chinook salmon from the third paired release, 1994.

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Section 8

THE EFFECT OF FEEDING SPRING CHINOOK SALMON A LIVE FOOD SUPPLEMENTED DIET DURING ACCLIMATION, 1995

by

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Contents

Introduction

A central question to increasing the postrelease survival of hatchery reared fish is determining if prerelease forage training increases postrelease foraging success. Many successful captive rearing programs for endangered and threatened species of higher vertebrates have successfully trained the animals to forage naturally prior to releasing them back into their natural environment (Beck et al. 1994). For instance, after research showed that cage-raised Siberian ferrets killed mice and prairie dogs more efficiently when they had previous experience (Miller et al. 1992), the captive breeding program for endangered black footed ferrets began providing captive bred animals the opportunity to stalk and kill live prairie dog (*Cynomys ludovicanus*) in large outdoor enclosures to develop their natural hunting skills prior to release (Oakleaf et al. 1992). Successful prerelease foraging training has also been conducted with Iberian lynx (Lynx *pardinus*) (Rodrigues et al. 1995).

Prerelease forage training has also been successful with cold blooded carnivores. Nile crocodile (*Crocodylus niloticus*) were successfully taught to forage naturally by being presented live fish in prerelease holding pools (Morgan-Davies 1980). Cultured chinook salmon (*Oncorhynchus tshawytscha*) exposed to a live food supplemented diet ate more live prey in laboratory test arenas than fish reared only on pellets (Maynard et al. 1996). Hybrid pike (Essox *lucius*) fed live foods had higher postrelease survival than fish reared on pellets (Johnson 1978)

Foraging training has also been implemented in captive breeding programs for herbivores. Prior to release, captive bred Golden lion tamarin (*Leontopithecus rosalia*) that were allowed to move around on natural vegetation and forage for hidden food in their cages developed better natural foraging skills. (Beck et al. 1991). In the case of avians, thick billed parrots (*Rhynchopsitta* pachyrhyncha) have been provided experience with handling their primary food source, pine cones, prior to reintroduction to the wild (Wiley et al. 1992). A unique tutoring program, in which nonendangered Texas bobwhite quail (*Colinus* virginanus texanus) were grouped with endangered masked bobwhite quail (C.*virginanus* ridgewayi) in acclimatization cages, was successfully used to demonstrate food-finding and antipredator behavior to captive bred animals (Carpenter et al. 1991).

In the study reported here, we examined how exposing spring chinook salmon to live food supplemented diets and a more natural rearing habitat during acclimation affected postrelease foraging ability. Both foraging theory and the above cited studies suggested these factors should enhance spring chinook salmon foraging ability.

Material and Methods

A 2 x 2 factorial design was used to examine the effect of live food supplemented diets and rearing habitat complexity on postrelease foraging ability. The four treatments consisted of 1) a gravel covered bottom and live food supplemented diet, 2) a gravel covered bottom and a pellet only diet, 3) a barren bottom and a live food supplemented diet, and 4) a barren bottom and pellet only diet. Habitat complexity of the rearing tanks was increased by covering the bottom with 2-cm gravel, so that fish could learn to forage in this more complex environment.

The experiment was conducted with 1993-brood Yakima River spring chinook salmon. Fish rearing was conducted at the National Marine Fisheries Service (NMFS) Freshwater Fish Culture Laboratory at the University of Washington's Big Beef Creek Research Station near Seabeck, Washington. The fish were fed a commercial semimoist diet and reared in a common

circular tank following standard fish culture practices. The experiment was initiated in March 1995. The yearling fish were anesthetized in MS 222, their fork length measured to the nearest mm, and PIT tagged. PIT tagging was done with an automated injector following the procedures outlined in Prentice et al. (1990). The tagged fish were randomly distributed to 24 400-L rectangular acrylic aquarium tanks.

The two ends and sides of each tank were covered with grey colored material. Black plastic aquaria hoods covered much of each tank's surface. Each tank was supplied with 4 L/min of food-free well-water through a 10-cm diameter opening in one end of the tank Water exited the tank through a similar opening in the other end. Six tanks were assigned to each of the four treatments, and the bottom of half the tanks covered with l-cm diameter gravel.

The fish in all 24 tanks received an equal volume of feed pellets each day. Those fish on a live supplemented diet were also given a ration of brine shrimp or tubifex worms prior to being fed pellets. The fish were maintained on these diets until tested in the experimental enclosures.

In April 1995, all fish were measured, photographed, and a subsample of three fish from each tank sacrificed for pathological analysis. The remaining fish were returned to their tanks and reared as previously described.

The foraging ability of a 'subsample of fish from each treatment was examined in test enclosures beginning in mid May 1995 for the marine tests and late May 1995 for the freshwater tests. Size classes were established based on length and a subsample of fish removed from each aquaria for testing in the in situ enclosures. Three fish from each tank were removed, placed in a common transport container, and transferred to a marine enclosure at the NMFS Manchester Marine Experimental Station. Another three fish from each tank were removed, assigned to a freshwater test cage, and transported in a common container to the Union River near Belfair, Washington, where they were released into their assigned test cage.

The marine enclosure was a 2-m wide by 3-m long fiberglass raceway. The bottom of the raceway was covered with several centimeters of pea gravel and the water level was maintained at 48 cm Unfiltered seawater flowed into the raceway at approximately 14 L/min The raceway was allowed to develop a natural flora and fauna over several months time before the test fish were added. A plankton bloom occurring in the surrounding waters enhanced productivity during the test period.

The six freshwater enclosures were 2.4-m long by 1.2-m wide by 1.2-m deep nylon net (1.9-cm stretch mesh) cages fitted around a PVC pipe frame. The top of each cage was covered with a l-cm thick sheet of plywood. The cages were anchored in place on the stream bottom by steel fence posts driven into the bottom and attached to each comer pipe. The six cages were dispersed along a 0.5-km section of the river.

After about 1 week of residence in the enclosures, the fish were netted, anesthetized in a lethal concentration of MS 222, length measured to the nearest mm, and scanned for PIT-tag code. The stomach contents of the fish were immediately preserved by formaldehyde injection of the stomach. After fixation, the stomach contents were transferred to a 70% ethanol solution for storage until analyzed. The stomach contents of each fish were sorted into digestible and indigestible material. The sorted material was then weighed to the nearest 0.001 g providing a measure of individual foraging success.

Contingency table analysis was used to statistically analyze the pathology data. A two way ANOVA was used to analyze the length and stomach content weight data.

Results

During the course of the study, in-culture mortality was relatively high ranging from 7.9 to 14.1% (Pig. 8-l). Although not statistically significant (P = 0.077), the in-culture survival of fish fed a live food supplemented diet was higher than that of fish fed pellets only. Additionally, fish whose diets were supplemented with live foods were significantly (P = 0.006) longer than fish fed a pellet only diet (Pig. 8-2). Rearing habitat complexity affected neither survival (P= 0.917) nor growth (P = 0.327).

Yersina ruckeri, the causative agent of enteric redmouth disease was not present in fish from either sample. The kidney tissue samples for determining the presence or absence of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), in fish from the rearing treatments have not been read at this time. However, gross autopsies of mortalities indicated that Renibacterium *salmoninarum* was the main cause of death for fish from all treatments.

In both test environments, most fish had little digestible material in their stomachs. In the marine enclosure, 85% of the fish had less than 0.1 g of digestible material (Pig. 8-3). In the freshwater enclosures, 49% of the fish had stomachs that were either empty or contained less than 0.1 g of food (Fig. 8-4). Less than 10% of the fish in either type of enclosure had more than 0.3 g of digestible material in their stomachs.

Rearing habitat complexity had no significant effect (P > 0.05) on foraging ability in either freshwater or marine enclosures (Pig. 8-5 and 8-6). In the marine enclosure, the fish from the live food supplemented diet had significantly less food in their stomach than pellet-fed fish (P = 0.035). Although not statistically significant (P = 0.095), the results were similar in the freshwater enclosures, with more digestible material in the stomach of fish from the pellet-fed acclimation treatments.

Discussion

In the wild, spring chinook salmon stomach contents usually weigh more than 1% of total body weight. With few exceptions, the stomach contents of chinook salmon captured in the intertidal area of the Nanaimo River estuary weighed 1-4% of the fish's body weight (Healey 1979). By the time the fish were tested in the enclosures, they were about 145 mm long, and at that size, we would expect digestible material in their stomach to weigh from 250 to 1,000 mg. As most of our in situ enclosure fish had less than 100 mg of digestible material in their stomachs, we believe they were not feeding as well as they should

In both types of enclosures, more food items generally preyed on by salmonids appeared to be available than were eaten during the test period. In the marine enclosure, plankton was so abundant that there was insufficient visibility for videotaping fish foraging behavior and amphipods were observed in the enclosure at the end of the test. In the freshwater tests, uneaten aquatic insect prey (such as stoneflies, *Plecoptera*) were observed within the enclosures when the fish were being removed at the end of the week. These observations suggest that it was failure of

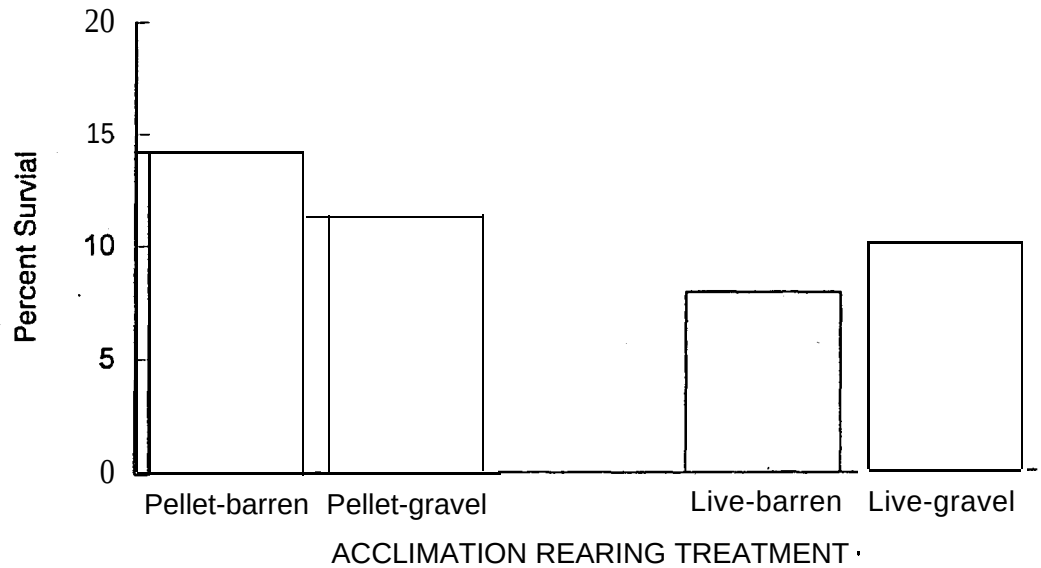

Figure 8-l. Percent in-culture mortality of 1993-brood Yakima River spring chinook salmon acclimated on pellet or live-food diets and structured or unstructured habitats.

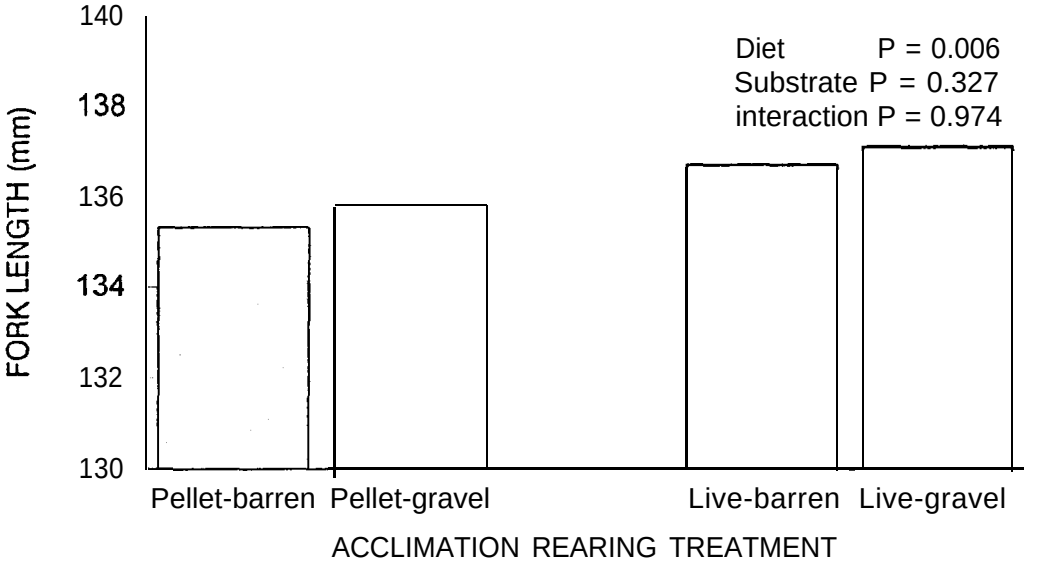

Figure 8-2. Fork length of 1993-brood Yakima River spring chinook salmon acclimated on pellet or live-food diets and structured or unstructured habitats.

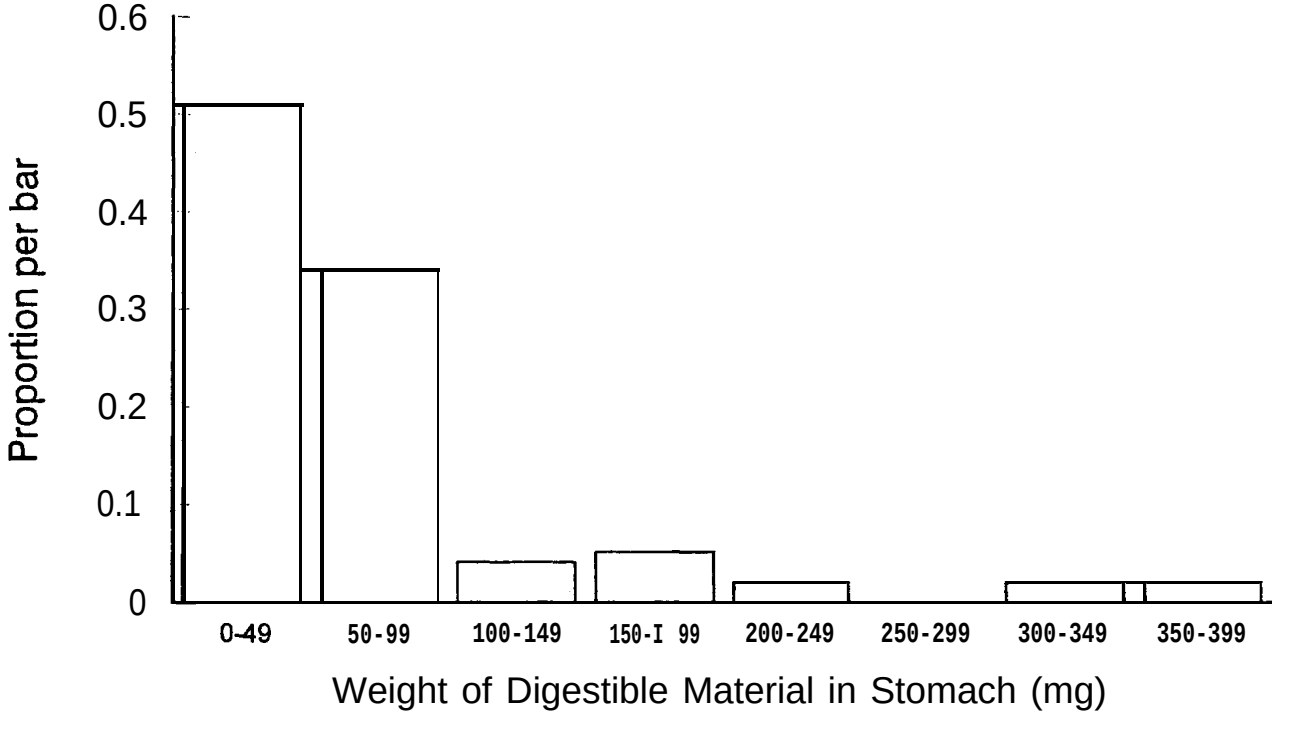

Figure 8-3. Weight (mg) of digestible material in stomachs of 1993-brood Ynkima River spring chinook salmon acclimated on pellet or live-food diets and structured or unstructured habitats and challenged to forage in a marine enclosure.

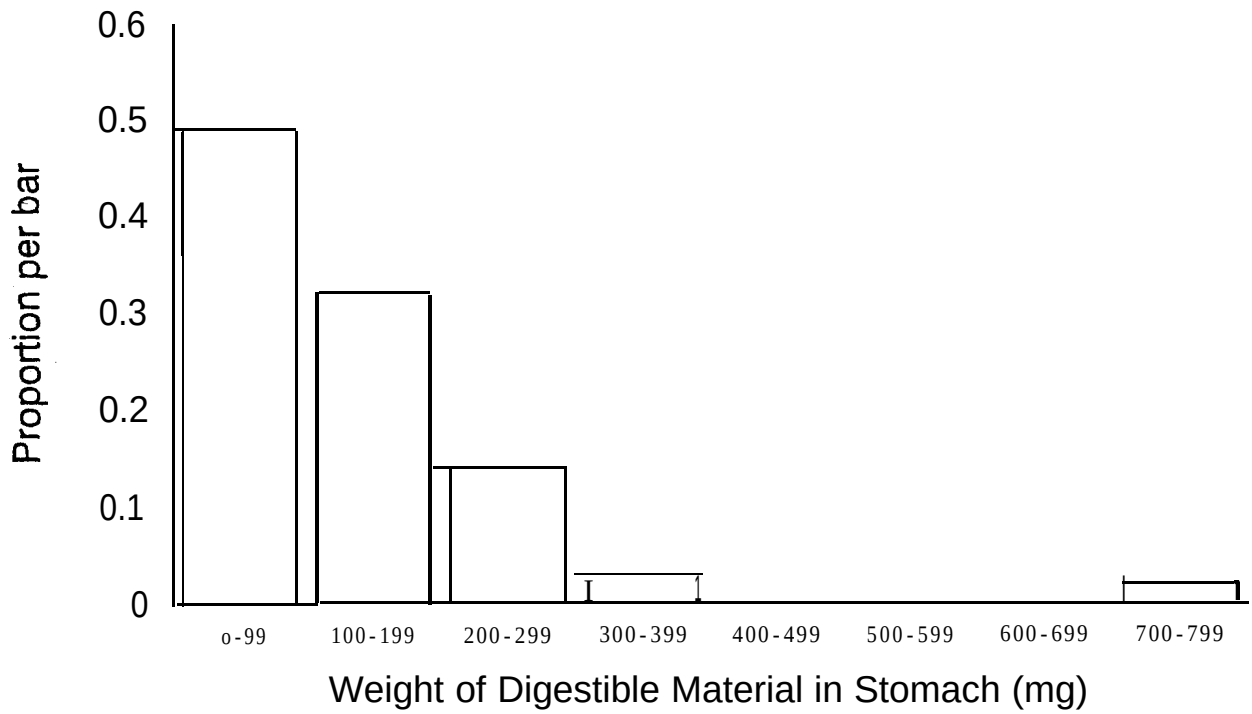

Figure 8-4. Weight (mg) of digestible material in stomachs of 1993-brood Yakima River spring chinook salmon acclimated on pellet or live-food diets and structured or unstructured habitats and challenged to forage in a riverine enclosure.

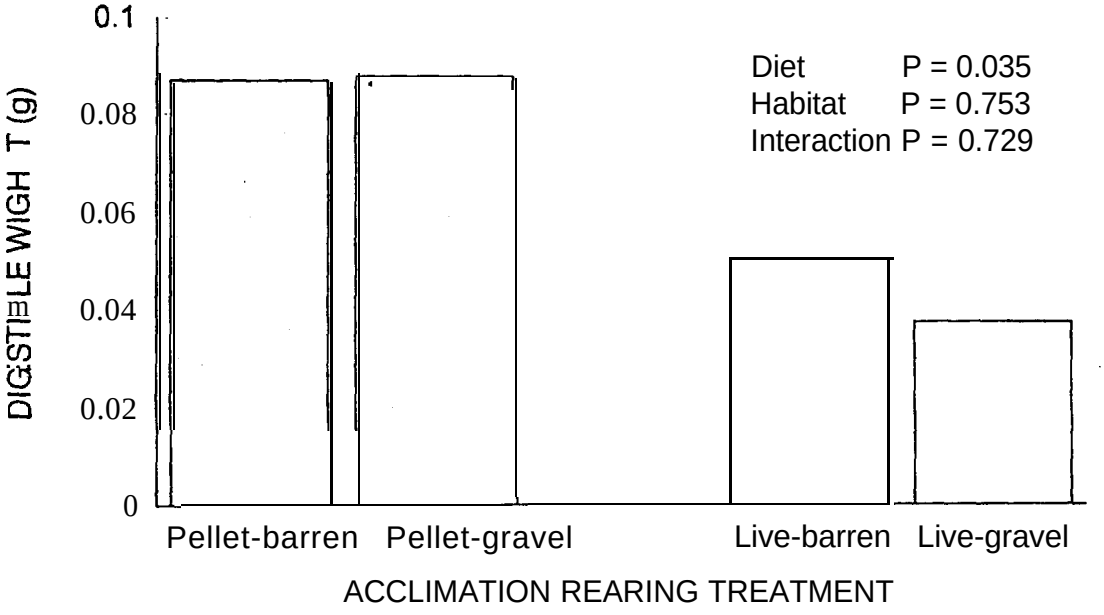

Figure 8-5.‘ Stomach content weight of 1993-brood Yakima River spring chinook salmon acclimated on different diets in different habitats and challenged to forage in a marine enclosure.

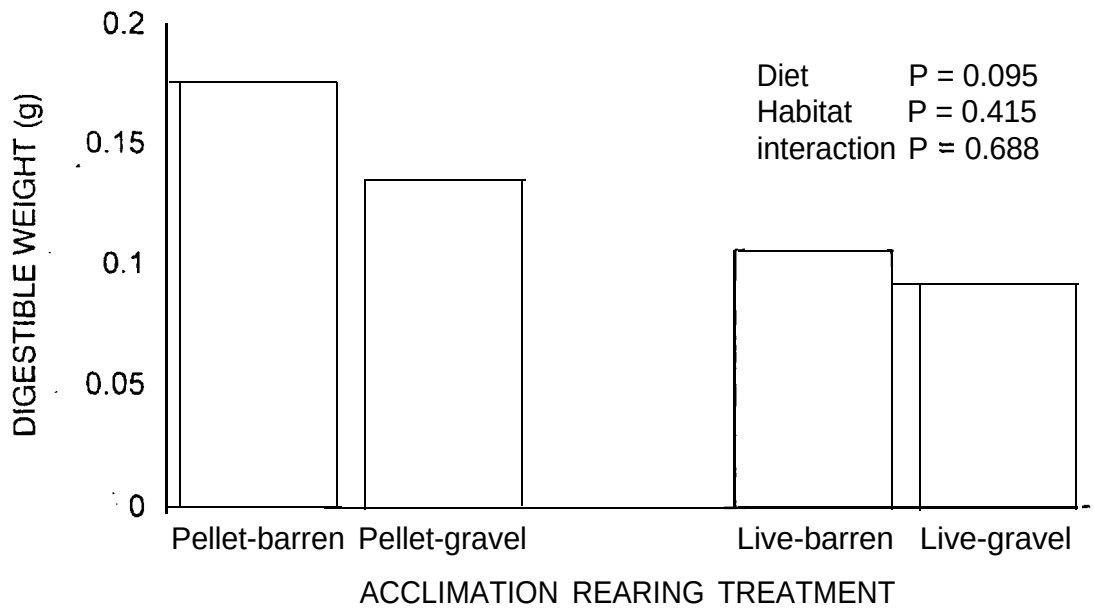

Figure 8-6. Stomach content weight of 1993-brood Yakima River spring chinook salmon acclimated on different diets in different habitats and challenged to forage in riverine enclosures.

fish to feed on the available prey in the enclosures rather than insufficient prey being available within the enclosures that resulted in so many fish with near empty stomachs.

In laboratory studies, more than a third of the hatchery-reared fish failed to feed even though food was plentiful (Paszkowski and Olla 1985, Maynard et al. 1996). In field studies, hatchery-mated fish are often found to be starving and have little or no food in their stomachs for the first few weeks after release (Miller 1952, Hochachka 1961, Reimers 1963, Sosiak et al. 1979, Myers 1980, O'Grady 1983, Johnsen and Ugedal 1986). The fact that a large number of hatchery-reared fish in many studies fail to feed demonstrates the need to develop culture techniques that improve the postrelease foraging ability of cultured fish.

Theoretically, the poor foraging ability of cultured fish may be attributed to 1) stress associated with entering a new environment, 2) disease, 3) inability to recognize live prey as food, 4) taste bias against live food, 5) inability develop successful hunting tactics, and 6) inability to switch to novel prey. In our study, the 1 week residence in the test enclosures should have provided sufficient time for the fish to recover from the effects of handling stress and begin feeding. Nevertheless, the fish in our study did not feed.

The high proportion of *Renibacterium salmoninarum* in the population does not explain the low stomach content weight of the test fish. When the stomach weights of fish showing gross symptoms of BKD were compared to fish not showing those symptoms, the stomachs of the former fish had a greater weight of digestible material. Although it is possible that sick fish do not have as rapid a gastric evacuation rate as healthy ones, this suggests that fish undergoing a BKD infection forage as well as healthy ones.

Salmonid foraging is a multi-step process involving prey detection, capture, and ingestion. Simple visual cues are the primary behavior releaser. Our observations are that during the prey detection process, salmonids pursue any object moving in their visual field that is small enough to engulf, including bubbles and vegetable matter. Visual and acoustic cues are then used by the fish to capture the prey. No discrimination occurs up to this point, with both digestible and indigestible material being pursued and captured with equal vigor. However, once the prey can be tasted and felt within the oral cavity, discrimination occurs and the prey is either ingested or rejected This model is based on several hundred hours of observing coho salmon and chinook salmon feeding behavior (D. Maynard, personal observation), and is supported by our earlier observation that chinook salmon repeatedly attacked and captured mayflies until they breached the exoskeleton and rejected the prey based on its taste (Maynard et al. 1996).

Based on this foraging behavior model, prey texture and taste are probably the most important factors involved in developing techniques to successfully condition hatchery-reared salmonids to forage on natural prey. Bryan and Larkin (1972), Ringler (1985), and Merna (1986) suggested juvenile salmonids develop their tastes for food early in their life cycle and that these tastes are then maintained throughout their life. This leads us to suggest that most pellet-reared fish ate not ingesting live prey in laboratory and field studies because they do not develop that initial taste for natural live foods early in their rearing cycle.

However, when fish are reared primarily on pellets, but are given early experience with natural live foods, they may develop a taste for the feeds they must consume after release. This concept is supported by our observation that hatchery fall chinook salmon exposed to large amounts of natural prey entering their rearing vessel throughout their hatchery rearing cycle seemed to feed readily after release (E. Tezak and D. Maynard, personal observation). This suggests that

future research should determine if feeding live-food supplemented diets fed from swimup to release is a better approach for enhancing postrelease foraging success of hatchery reared fish.

It is unclear why fish in this study reared on a pellet-only diet contained mote digestible material in their stomach than fish reared on live food supplemented diets. Both theory and our earlier investigation with fall chinook salmon suggested the foraging experience provided by live food diets should improve the foraging skills of spring chinook salmon over that observed with fish reared on pellets alone.

One explanation is that the color and shape of feed pellets more closely resembled the natural prey available in the enclosures than the brine shrimp and blackworms used in the live food supplemented diets. However, since fish from both treatments had similar experience with pellets, they should have developed identical search images for pellet-like prey.

The difference between the two treatments might also be explained if the fish reared on live-food supplemented diets had more rapid gastric evacuation rates than fish reared on pellets alone. As reviewed in De Silva and Anderson (1995) both the type of food present in stomachs and the length of time since last feeding affects gastric evacuation rate. The gastric evacuation rate is faster for fish feeding on smaller particles than large particles, less fatty foods than more fatty food, and invertebrates with thinner exoskeltons than thicker exoskeltons.

If the live food supplemented fish were more prone to feed on smaller prey, prey without exoskeltons (worms), or less fatty prey, than their stomachs might contain less material at sampling because they were evacuated more rapidly. Similarly if it took the fish reared on pellets alone several days longer to learn to capture live prey, their stomachs might contain more food, as fish that have not fed for some time have gastric rates 50-68% slower than fish that have been continuously feeding (De Silva and Owoyemi 1983).

The live food dietary supplements did improve in-culture survival. Micronutrients or vitamins present in the live foods may have enhanced the performance of the immune system of fish teared on live food supplemented diets. Research has shown that higher dietary levels of Vitamin C than is found in standard prepared diets enhances the immune system of salmonids (Verlhac and Gabaudan 1994).

Given observations with other species, it is surprising that habitat enrichment in this study had no effect on postrelease foraging ability. However, this observation may be the result of very few fish in the study feeding. For habitat enrichment to enhance foraging ability, it may first be necessary to instill a preference for live food diets in salmonids. Once a dietary protocol is determined that results in most fish successfully foraging, then research should again be initiated to determine if habitat enrichment also enhances postrelease foraging ability.

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Section 9

INSTREAM POSTRELEASE GROWTH AND SURVIVAL OF CHINOOK SALMON SMOLTS SUBJECTED TO PREDATOR TRAINING AND ALTERNATE FEEDING STRATEGIES, 1995

by

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Contents

Introduction

Anadromous salmonids often suffer high mortality after being released from hatcheries. Predation can be a major source of mortality for juvenile salmonids and may be particularly intense on hatchery-reared fish that have incomplete development of antipredator behaviors (Olla and Davis 1989, Berejikian 1995). Naturally occurring fish predators on juvenile salmonids include other salmonids, e.g., Arctic char, *Salvelinus aplinus*; steelhead trout, *Oncorhynchus mykiss*; cutthroat trout, 0. *clarki*; and coho salmon, 0. *kisutch* (Meacham and Clark 1979, Fresh and Schroder 1987, Beauchamp 1990, Ruggerone 1992), as well as non-salmonids, e.g., sculpins, *Cottus* sp.; and squawfish, *Ptychocheilus* sp. (Ricker 1941, Beall 1972, Patten 1975). The rate of predation by piscine predators on juvenile salmonids is regulated by a host of interacting environmental and biological factors (Ginetz and Larkin 1976, Ruggerone and Rogers 1984).

Juvenile salmonids have innate antipredator responses that can improve with experience. Predator avoidance ability of juvenile salmonids improves after exposure to piscine predators (Ginetz and Larkin 1976, Patten 1977), and learning in particular may play an important role in predator avoidance ability for chinook salmon (0. *Tshawytscha*), coho salmon (Thompson 1966, Olla and Davis 1989, Healey and Reinhardt 1995) and steelhead trout (Berejikian 1995). Typical hatchery rearing environments possibly obscure the development of anti-predation responses because hatcheries lack sensory stimuli associated with predation (Thompson 1966, Olla and Davis 1989). Thus, reduced susceptibility to predators after release may result if juvenile hatchery-reared salmon are provided predator stimuli.

There is substantial evidence that fish, including juvenile salmonids, "trade-off' the energetic benefits of foraging with its associated costs, namely, increased vulnerability to predation (Dill and Fraser 1984, Gilliam and Fraser 1987, Magnhagen 1988, Abrahams and Dill 1989). The decisions regarding where, how, and how much to forage are also dependent upon the internal motivational state of the fish. Hungrier fish are more willing to accept greater risk to obtain food than satiated, or less hungry fish (Magnhagen 1988). Engaging in risky behaviors such as foraging increases a fish's vulnerability to predators (Gilliam and Fraser 1987) because, like all vertebrates, fish cannot be visually attentive to mom than one activity at a time (e.g., foraging and vigilance; Lima and Dill 1990).

Instream postrelease survival of chinook salmon smolts has been estimated to range from a low of 24.6% over a 225 km migratory corridor to 53.3% (2.2 km corridor) in three independent studies (Sections 5 - 7 in this report). Survival differences between experimental treatments in these studies occurred within a week or so after release, suggesting that predation may he the primary source of mortality rather than slower acting factors such as starvation or disease. The present study was designed to test the hypotheses that exposing hatchery-reared fall chinook salmon to a, piscine predator prior to release will improve their postrelease survival and that hungrier fish will suffer greater mortality than less hungry fish due to increased vulnerability to predators.

Materials and Methods

Study Site

This study was conducted at the University of Washington's Big Beef Creek Research Station near Seabeck, Washington. The study utilized Big Beef Creek, which enters Hood Canal about 4.5 km north of Seabeck. Stream flow during the study period was approximately 0.1 m^3/s. The main piscine predators in the stream are cutthroat and steelhead trout (Fresh and Schroder 1987). A weir capable of capturing 100% of emigrating smolts exists at the stream's entrance to the estuary.

A population of fall chinook salmon (originated from the Deschutes River, WA, population) has been perpetuated by spawning adults, then rearing and releasing 3 month-old smolts. The subjects used for this study were progeny of 11 females and at least as many males spawned over several weeks in October 1994. Fish were incubated in Heath trays and reared in 7.3-m diameter circular fiberglass tanks in 10°C ±0.5°C well water. One thousand three hundred fry were removed from the tanks and placed into a single, 1.8-m diameter tank with approximately 30 L/min inflow on 12 April 1994, where they were fed one to four times daily for a total daily ration of 1.5 to 2.8 body weight, All fish were injected with Passive Integrated Transponder (PIT) tags on 19 May 1995 and returned to the single rearing vessel.

Procedures

Two hundred fish were removed from the rearing tank at 1400 h on 15 June 1995 (day 1) and 100 fish were placed into each of two 0.75-m tanks receiving a flow of 15 L/min. On days 2 and 3, one tank was fed a ration equal to 2.0% of fish biomass (the "fed" tank) between 0800 and 1000 h and the other tank was not fed ("starved" tank). At 1200 h on day 3, alternate groups of 10 fish from the fed and unfed tanks were anesthetized with MS-222 and the individual PIT codes were recorded along with fish weight (to the nearest 0.1 g) and fork length (to the nearest 0.5 mm) until all 200 fish (100 from each tank) were processed. Within each group of 10 fish, individuals were alternately allocated to one of two identical 2.2-m diameter circular tanks. One tank received two predatory cutthroat trout 2 hours later, and the other tank received no cutthroat. The chinook salmon smolts were left in the "predator" and "control" tanks for 16 hours (1500 h to 0700 h the next day). All fish were then placed in a common 25 L transport tank and transported to a release site located at river kilometer 5.2 (Rkm) of Big Beef Creek. Hence, at the time of release, fed fish had been without food for 22 hours and unfed fish had been without food for 72 hours.

The entire process (i.e.; reading tags, feeding, predator exposure, and release) was repeated on 5 consecutive days, such that six releases were made at 0830 h from 17 June to 22 June 1995. The proportion of fish recaptured at the weir were analyzed by a randomized block (without replication) two factor analysis of variance (ANOVA) where feeding and predator exposure were the treatment effects and release day was the block effect. This analysis assumes no significant block by treatment interactions (Sokal and Rohlf 198 1).

Because fish were individually PIT tagged, we were also able to test for differences in growth and weight loss, changes in condition factors, and travel time to the weir for individual fish. These factors were analyzed by a randomized block ANOVA with replication, where release date was the blocking factor. We also recorded the frequency of predatory "bite marks" on chinook salmon smolts recaptured at the weir.

Results

Predator Training and Feeding

Cutthroat placed in the "predator" tanks ate between one and six chinook salmon smolts depending on the release day. Similar numbers of fed and starved smolts were eaten during predator training (Table 9-l). The 2 days of feeding prior to release produced fed fish that weighed more ($P = 0.044$) and had a higher mean condition factor ($P = 0.014$) than starved fish at release, but the fed and starved fish recaptured at the weir did not differ in either weight or condition factor ($P > 0.05$ in both cases). Hence, food in the digestive system probably accounted for much of the difference in weight at release.

Postrelease Survival

There was no significant effect of predator training ($P = 0.99$) or feeding regime ($P = 0.68$) on the proportion of chinook salmon smolts recovered at the Big Beef Creek weir (Fig. 9-1). However, the proportion of fish recaptured differed by release day (Chi-square = 20.2, 5 df, P c 0.001). The proportion of fish recaptured decreased on each successive release day and substantial differences in recoveries by release day had occurred by 8 days after release (Fig. 9-2).

The average release weight and length of fish increased from release days 1 through 5 (Fig. 9-3). Within individual release groups, however, neither the release weight nor release length of survivors recovered at the weir differed from the average release lengths and weights of those fish not recovered (two sample t-tests, 1 df, $P > 0.05$ in all cases), indicating no size-selective mortality within a release group.

The proportion of predator-marked smolts (determined by the presence of bite marks) from a given release (all treatments combined) was inversely related to the proportion of smolts recovered at the weir from the same release ($F_{1,4} = 18.8$, $P = 0.012$, Fig. 9-4). This suggests that the number of attacks by piscine predators may have been greater on later release groups, assuming that the capture efficiency (i.e., proportion of successful attacks to total attacks) of the predators did not decrease over time (Donnelly and Whoriskey 1993). The ventral orientation of the fine "rake" marks on the smolts appeared to have been caused by the small sharp teeth of a salmonid predator. Only one smolt appeared to have been injured by an avian predator.

Changes in Body Size

Fish from later release groups grew (fork length) more than fish from earlier release groups from the time they were released until the time they were captured ($P = 0.034$). There was no difference in mean travel time to the weir by release date ($P = 0.212$), hence fish released on later days grew at a faster rate than did fish released on earlier days.

On average, chinook salmon smolts (all release groups combined) lost weight over the first 2 weeks after release. The lowest mean weight at recapture as a proportion of release weight (recwt/relwt) occurred 15 days after release. Over the subsequent 3 weeks until the final collection, recwt/relwt steadily increased (Fig. 9-5).

Table 9-l. Number of fed and starved chinook salmon smolts eaten by cutthroat trout in the predator training tanks, and the number of smolts (fed and starved combined) eaten by the smaller (< 275 mm) and larger (> 275 mm) of the two cutthroat trout, 1994.

	Total chinook salmon smolts eaten		Total chinook salmon smolts eaten, by predator size-class					
			Cutthroat trout < 275 mm			Cutthroat trout > 775 mm		
Release	Fed	Starved	Length (mm)	Weight (g)	Smolts eaten	Length (mm)	Weight (g)	Smolts eaten
1	1	0	234	117	0	278	208	1
2	3	3	264'	145	1	310	218	5
3	3	0	245	118	0	304	260	3
4	1	0	204	79	1	330	310	0
5	0	2	228	108	1	362	367	1
6	1	3	236	107	1	310	218	3
Total	9	8			4			13

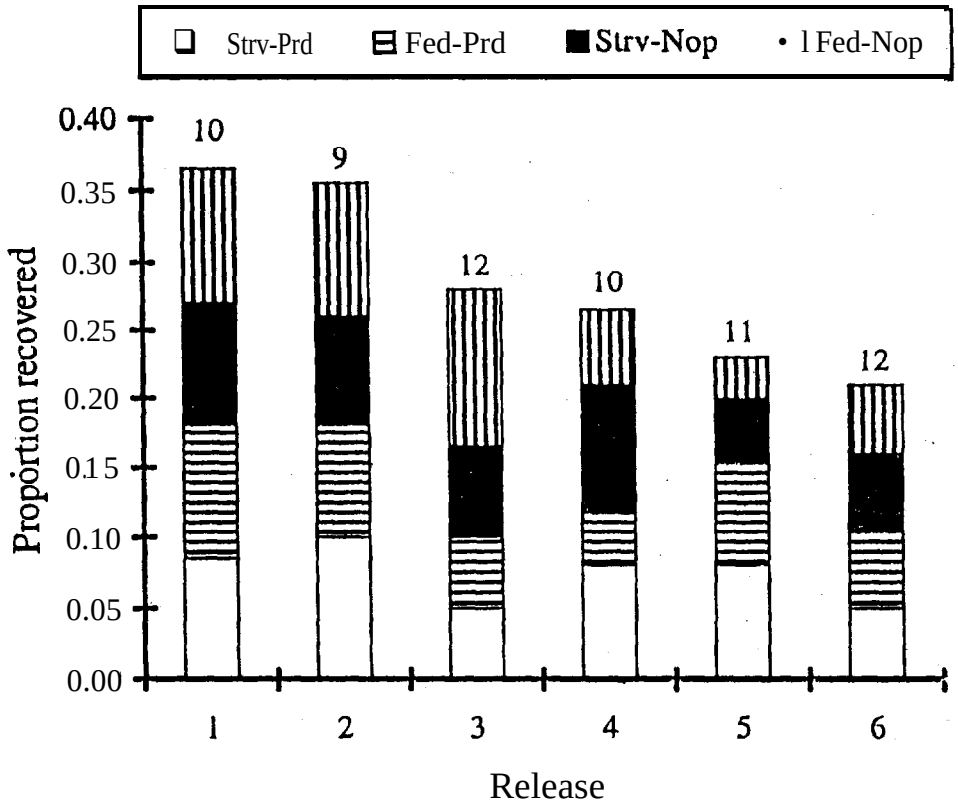

Figure 9-1. The total proportion of chinook salmon molts recovered at the Big Beef Creek weir that were fed and predator trained (Fed-Prd), fed and not trained (Fed-Nop), starved and trained (Strv-Prd), and starved and not trained (Strv-Nop). There was no effect of predator training (P = 0.99) or feeding regime (P = 0.65) on the proportion of smolts recovered. There was a significant effect of release group on the proportion of fish recovered at the weir (P = 0.001). The mean travel times in days to 50% recovery are shown above the bar for each release group.

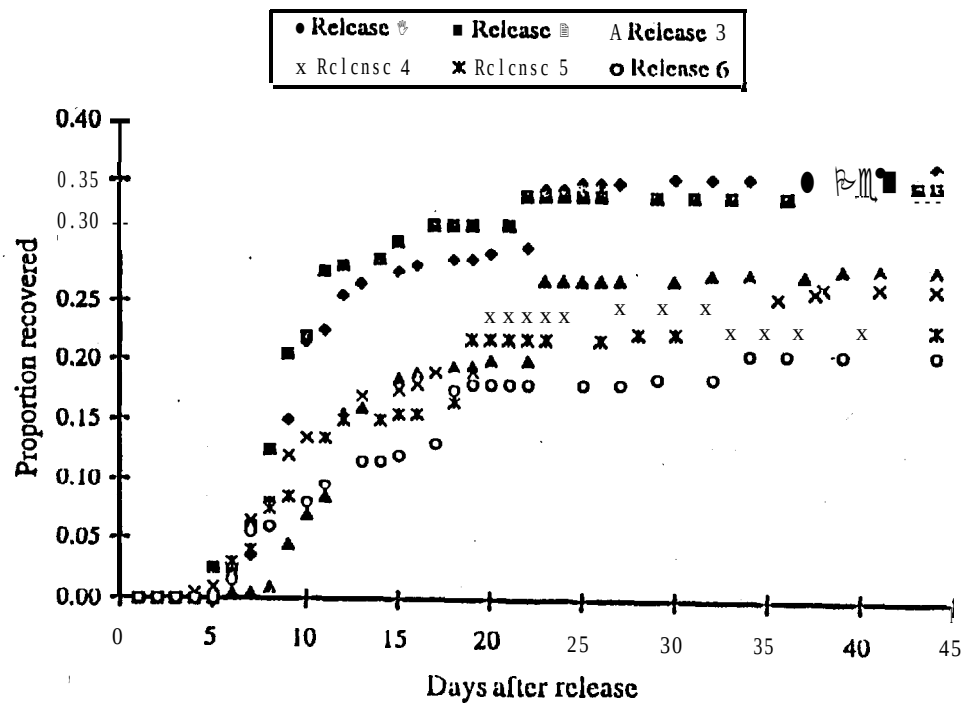

Figure Y-2. Cumulative proportion of chinook salmon smolts recovered from each release (all treatments conibined). Note that large differences in recoveries among release groups had occurred by 8 days post release.

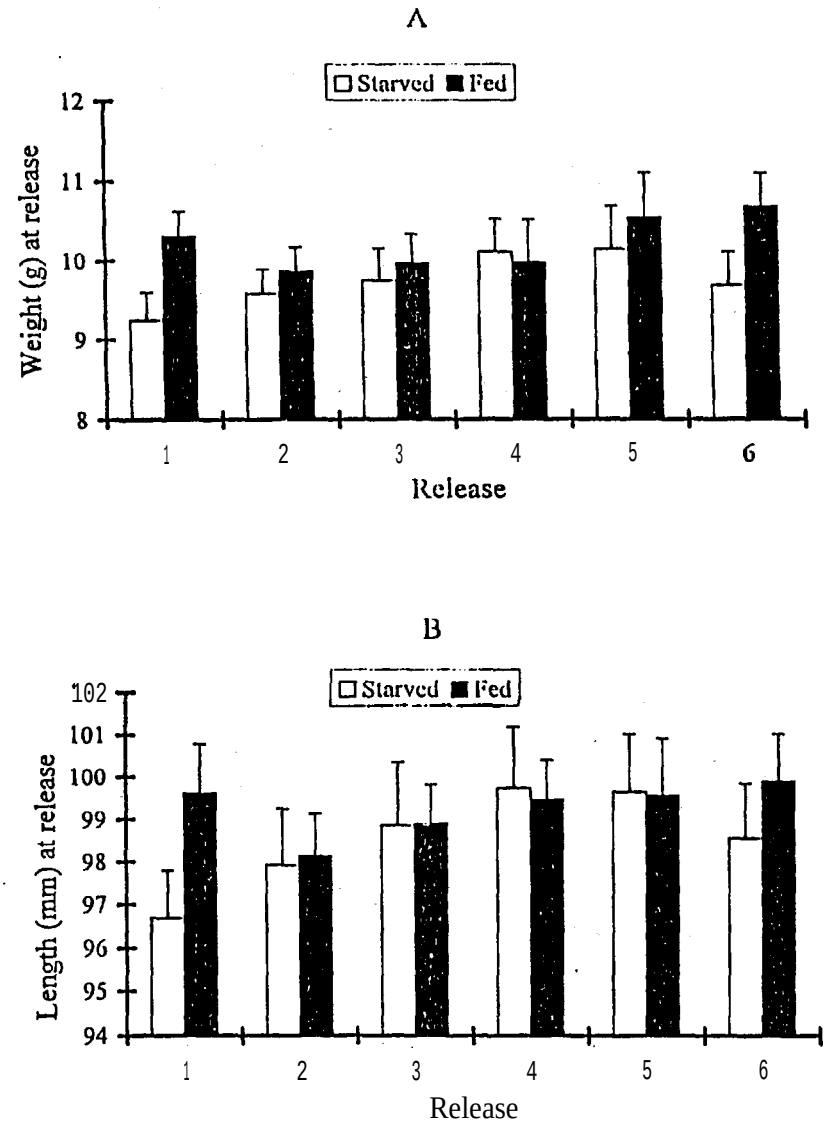

Figure 9-3. Average (A) weights and (B) fork lengths (+/- s.e.) of fed and starved chinook salmon smolts recorded 1 day prior to release.

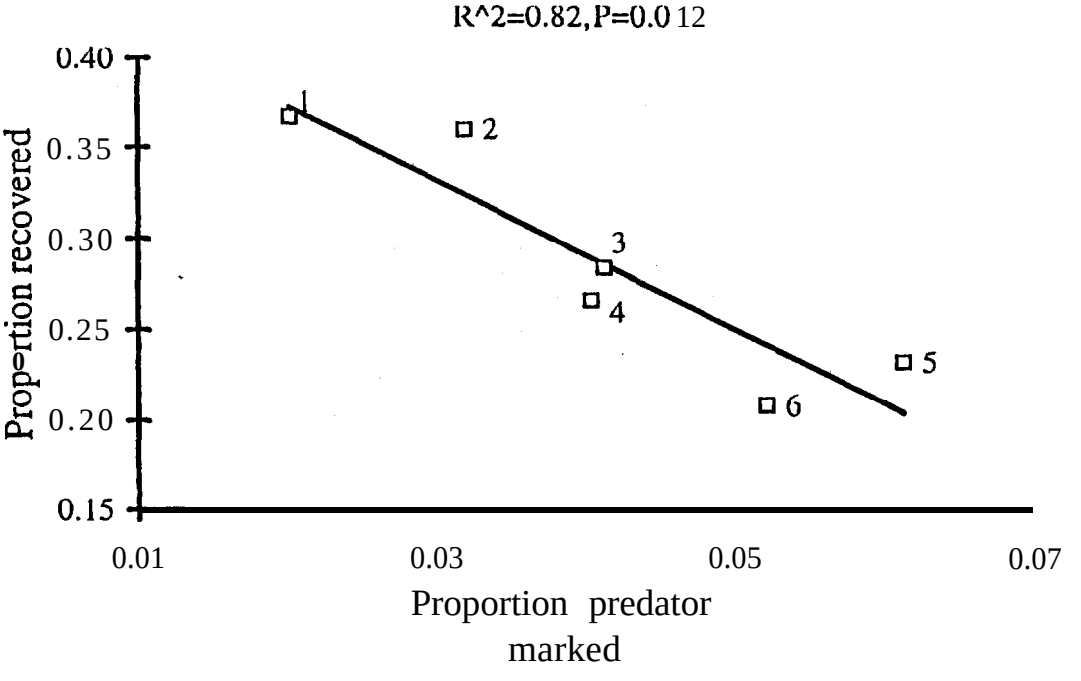

Figure 9-4. The significant linear relationship (P = 0.012) between the proportion of chinook salmon molts recovered (both treatments combined) on a given release day and the proportion that were recovered with predator marks. Release days are shown next to individual data points.

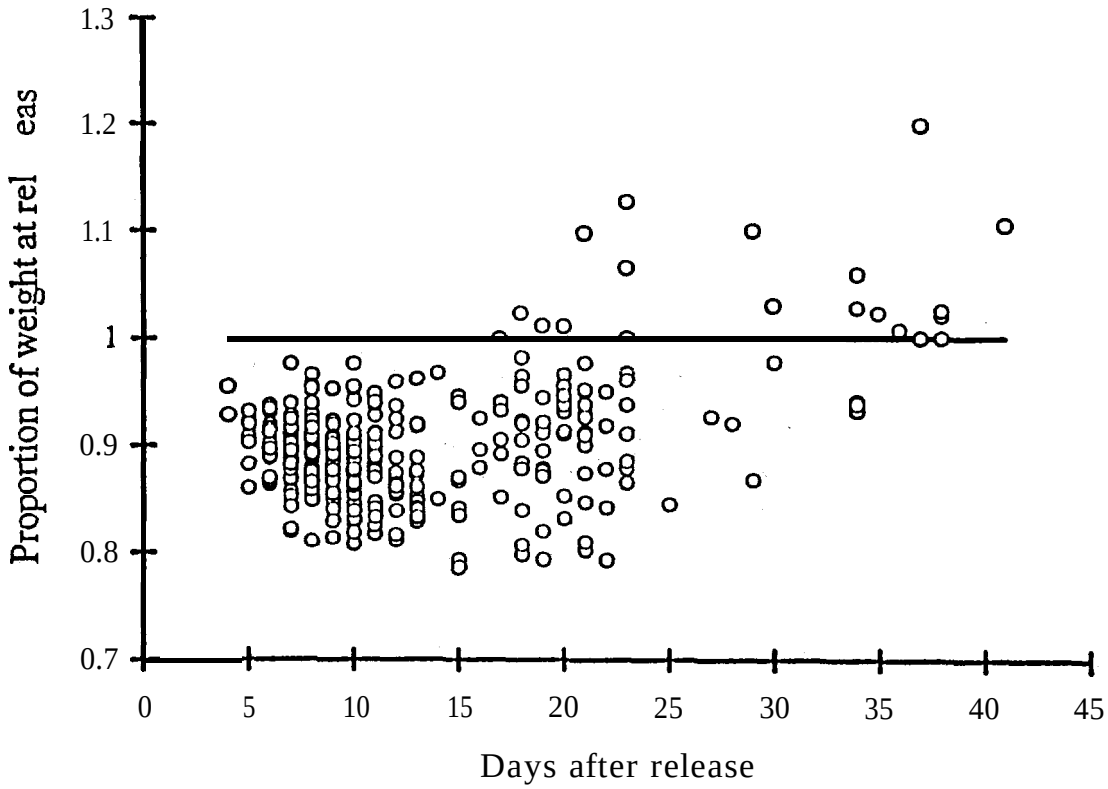

Figure 9-5. The ratio of release weight to recovery weight for 341 chinook salmon molts (both treatments and release groups combined) as a function of travel time to the Big Beef Creek weir. The horizontal line represents a ratio of 1 (i.e., no change in weight).

Discussion

The effect of hunger on predator vulnerability would have been most evident during the first few days after release because after about 2 days, the amount of food in the gut (probably the best measure of hunger, Dill 1983) of fed and starved fish would have been about equal. No fish migrated within 2 days, hence the effect of hunger was probably equalized between groups beyond 2 days for the duration of downstream migration, and may have masked any differential survival during the first 2 days after release.

It is possible that the antipredator training procedure used in this study was not extensive enough to improve antipredator recognition or anti-predation responses for the chinook salmon smolts. However, several studies have noted an increase in predator avoidance ability after only briefly exposing prey to predators. Olla and Davis (1989) trained coho salmon to avoid lingcod after two, 15 minute exposure periods. Berejikian (1995) found that steelhead fry exposed to visual predation by sculpin on other steelhead for 50 minutes had an effect on their subsequent predator avoidance ability. It took only two captures by rainbow trout of chinook salmon and coho salmon fry to alter these prey's antipredator behavior (Healey and Reinhardt 1995).

Other studies have also shown that prior exposure improves subsequent predator. avoidance ability (Ginetz and Larkin 1976, Patten 1977). However, little evidence exists that predator training has improved postrelease survival of salmon smolts into a natural stream. Although Thompson (1966) found higher postrelease survival to a weir for chinook salmon that had been trained with electrified fish models compared with those that had no training, the experimental design precludeda valid statistical evaluation of the experiment. Therefore, although the aforementioned laboratory studies demonstrate the learning ability of juvenile salmon to avoid predators, the relevance of these studies to actual increases in postrelease survival has yet to be established.

The proportion of fish recovered at the weir declined for each successive release day. Increased piscine predator activity, indicated by the increase in the proportion of fish with bite marks on successive release days, may have been partly responsible. A numerical response (Hunter 1959) of avian predators to the increase in available prey may also have contributed to the poorer survival of later release groups.

Predation by avian predators may have masked any potential differences that may have existed in the ability of trained and untrained fish to recognize and respond to predatory cutthroat trout. In particular, belted kingfishers (*Ceryle alcyon*) were abundant in the study area and were observed feeding on salmonids throughout the 5.2 km stream section. Although the lack of avian predation marks on chinook smolts captured at the weir may indicate that birds were not significant predators, they may simply have had a greater success rate than piscine predators, particularly if the piscine predators were gape-limited and were able to capture chinook salmon smolts but not able to ingest them. On 28 July 1995, we counted (by snorkeling) 31 cutthroat trout with estimated lengths greater than 200 mm in Big Beef Greek from the weir upstream to about Rkm 1. Data from the predator training tanks demonstrates that cutthroat larger than 275 mm captured more smolts than those cutthroat smaller than 275 mm (Table 9-l). Because the majority of cutthroat trout in Big Beef Greek were estimated to be shorter than 275 mm, their capture efficiency on chinook salmon smolts (average fork length = 98 mm) may have been quite low.

Kingfishers are homeothermic and therefore have much higher rates of metabolism than salmonids. Hence, they have the metabolic capability of consuming far greater numbers of smolts per predator per unit time. Gastric evacuation rates of salmonids, which limit their rate of food

intake (Ruggerone and Rogers 1984, Ruggerone 1989), are very slow (e.g., approximately 2 days for *O. mykiss* at 10°C: Beauchamp 1990) compared to birds, which can process a substantially greater amount of food per unit time (cf. Wood 1987). We believe that kingfishers probably consumed far greater numbers of chinook salmon smolts than did cutthroat, although we have no data to support this claim. Future studies will focus on successfully training salmon smolts to avoid the most significant predator(s) they are likely to encounter after release, which, depending on the postrelease environment, may include piscine, avian, or even terrestrial predators.

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Section 10

REVIEW OF FISH MARKING AND TAGGING PROCEDURES SUITABLE FOR THE NATURES PROGRAM

by

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Introduction

As discussed in previous sections of this report, the NATURES program is an attempt to improve hatchery practices to produce physiologically and behaviorally competent, healthy fish. The NATURES program addresses that need and is an embodiment of a common sense approach to raising fish--one that examines the biological needs of the cultured organism and uses that information to provide optimal rearing habitats and behavioral experiences.

For the NATURES approach to work, two questions must be answered: The first simply asks whether alterations to existing hatchery conditions will produce salmon with attributes that accentuate their postrelease survival. The second is whether the physical and operational changes recommended by the NATURES program can be instituted in a cost-effective way. Comprehensive studies that rely on our ability to identify fish originating from different environments or treatments must be conducted to answer these questions.,

We believe the NATURES program has three major fish marking or tagging needs. The first is to apply visible marks to small salmonids so behavioral comparisons can be performed among fish that have undergone different early-life rearing treatments. The second is to use internal or visible marks on fish to assess early postrelease survival, migration patterns, habitat preferences and so on. The third is to provide either external or internal tags that can be applied at juvenile stages and deciphered at adulthood.

This third type of tag fulfills two intertwined needs: the need to assess overall survival and the need to evaluate how rearing conditions may affect parameters linked to potential fitness. Changes in fecundity, egg size, migration timing, and age-at-maturation are examples of the types of attributes that could be appraised by monitoring tagged adult fish.

In this review, we address the first marking need: to identify techniques that can be used to place visible marks on small (50 to 100 mm) salmonids. We found a number of potentially useful procedures and overviews of each method follow. In some instances, we proposed modifications to these procedures.

While conducting a literature review, it became clear that the underwater visibility of marks has never been quantitatively assessed. Moreover, how visible marks may influence the behavior of tagged fish, unmarked conspecfics, and potential predators has rarely been ascertained. These factors are important in the NATURES program because mixtures of juvenile salmon will be observed in situ to ascertain whether various rearing conditions create fish with differing behavioral characteristics.

Mark visibility and behavioral effects are difficult to measure under natural conditions. For this reason, we designed and built a portable viewing chamber where such observations could take place. The visibility and longevity of a number of promising marks were evaluated in this chamber. A description of the chamber plus some of our preliminary mark evaluation data are provided in the last portion of this review.

Selection of Marking Techniques

A number of criteria such as study objectives, behavioral effects, mark permanency, number of individuals to be marked, information content required of a mark, stress caused at the time of marking, and skill required to apply marks must be considered when determining which

techniques should be used in marking and tagging studies (Wydoski and Emery 1983). Foremost among these is deciding what type of information is required from the marked fish.

One of the primary goals of the NATURES study is to ascertain how alterations in rearing environments affect the behaviors of juvenile fall and spring chinook salmon, coho salmon, and steelhead. These species were selected because they are targets in a major supplementation and enhancement effort planned for the Yakima River Basin. For this effort, fish will be reared in raceways and then transferred to acclimation ponds, where they will be fed until liberation.

Three cultural strategies will be used for the Yakima studies: some fish will be reared under standard hatchery conditions throughout their entire cultural lives (the "standard treatment"). Others will be placed into raceways and acclimation ponds that have been modified to create fish with wild-like characteristics (the "Yakima treatment"). A third treatment group will experience the standard treatment during the raceway period of their life but receive the Yakima treatment once they have been introduced into an acclimation pond (this is termed the "mixed treatment"). For fall chinook salmon, the entire cultural experience will take place over a 90-to- 120 day period. Coho salmon and spring chinook salmon will be held for 1 year while some steelhead may be reared for 2 years.

Given the rearing treatments described above, we established the following minimal characteristics for visible marks:

1). **Permanency:** Fish, particularly those $\leq$ 50 mm long, may require up to 6 weeks to recover from stresses associated with marking. Consequently, marks should last long enough for fish to exhibit typical behaviors, or approximately 60 days. This minimal period of time should provide opportunities for behavioral comparisons among treated groups placed in a common environment. An ideal mark would be discemable throughout the entire freshwater rearing period and at the adult stage as well.

2). Underwater visibility: The visibility of a mark is largely dependent on its size, location, shape, and color, as well as environmental parameters like light intensity and water turbidity. Since observations will predominately occur in viewing chambers that mimic rearing habitats, light intensity and turbidity will often be controlled. However, it is possible that some fish will be examined in acclimation ponds and natural stream areas. In all of these settings, observers should be able to accurately classify marks when fish ate 1 to 3 meters away.

3). Behavioral impacts: It is imperative that visual marks have a neutral effect on the fish. -For instance, the social status, foraging capacity, and susceptibility to predation should not be altered by a mark. Moreover, marks must not create a physiological drain on the fish, (e.g., by impeding their maneuverability or by creating a chronic wound).

4). Informational content: The treatment array demands that at least three distinct forms (i.e., shapes, colors, locations, etc.) of a mark must be available. A very desirable trait for a mark is for it to be undecipherable while a fish is being observed yet readable afterwards. Few visible marks have this capacity, yet such marks help alleviate any unintentional biases an observer may have. Consequently, while reviewing the marking literature, we continually kept in mind how existing marking methods might be altered to provide this characteristic.

These criteria were used to screen existing tagging and marking techniques. Of the many methods available, four (branding, laser marking, V.I. tags, and panjetting) were chosen for in-depth review. Two commonly used procedures, mutilation and external tags, are not included in this review.

Mutilation, which generally consists of fin-clipping, was discounted as a possible technique for two reasons.

First, fins have prominent functional roles: they are obviously used for propulsion, to control pitch, yaw, and roll, to maintain water-column position, and for rapid and fine-scale movements (Harris 1936, 1937, 1938; Alexander 1970). Fin color patterns and movements are also used as signaling devices in fishes (Baerends and Baerends-Van Roon 1950, Reimers 1968, Stein et al. 1972, Swain and Riddell 1990).

Second, a great deal of variation in post-marking survival has occurred in fin-clipped fish (Bergstedt 1985). For instance, Nicola and Cordone (1973) concluded that fin removal had serious detrimental impacts on fmgerling rainbow trout. Yet data collected by Nielson et al. (1957) on the same species indicated fin-clipped fingerlings survived as well as unmarked cohorts. Because of the functional importance of fins and the uncertain effects caused by their removal, fin excision was not considered to be an acceptable visual marking procedure for the NATURES program.

External tags were also deemed inappropriate because losses can be high and such tags are known to attract predators, interfere with locomotion, reduce growth and survival, and cause chronic wounds (Wydoski and Emery 1983, Zak 1984, Buckley and Blankenship 1990, Haw et al. 1990, Bergman et al. 1992). Moreover, the histopathology of external tags reduced the likelihood of normal behavior and long-term data recovery (Buckley and Blankenship 1990). These effects are largely caused by how the tags are attached.

Characteristically, external tags have an external part attached to an anchor, which either passes completely through the fish or is lodged in subdermal tissue (Buckley and Blankenship 1990, Bergman et al. 1992). Currents, the presence of sessile organisms on the exposed surfaces of the tag, and the formation of scar tissue and normal growth around the anchor, continuously apply shearing and tearing forces on the tag. This relentless irritation prevents healing, and tagging incisions often become chronically inflamed and infected (Buckley and Blankenship 1990, Haw et al. 1990). Bergman et al. (1992) stated that a good solution to this percutaneous problem is doubtful, even when biocompatible materials and relatively small tags are used. Consequently, at least for the present, such tags should not be used in the NATURES program.

As mentioned above, four general marking/tagging procedures were identified as potential techniques that could be used to mark small salmonids. In the sections that follow, each method is described, and when applicable, suggestions on how it could be modified to more fully achieve the evaluation goals of the NATURES program are also included.

Overview of Select Marking Procedures

Branding

Historical Development of Brand Marking Methods

For the past 40 years, electrical and heat-transfer procedures have been used to brand fish. The first reported use of the method was by Buss (1953) who used an electric wood-burning pencil to mark young brook trout. He reported that fish retained brands for 1 to 2 years. Johnson and Fields (1959) and Watson (1961) heated a short coil of nichrome heating-element wire until it became white hot; the wire instantly burned through scales and skin, leaving black marks.

Watson (1961) burned a series of fine parallel lines on the lower anterior surface of sea herring, These lines disappeared in 2 to 3 days, but formed a dark oval patch, which was recognizable for 7 months. Johnson and Fields (1959) noticed that the brands they placed on juvenile steelhead were slow to heal and that they were no longer detectable after 5 months.

An important innovation in the technique was reported by Groves and Novotny (1965). They initially attempted to brand juvenile salmon with a small electrical soldering iron but were not satisfied with the appearance of the marks. Instead they made brands from pencil-sired copper tubing, each with a silver tip containing a symbol. These were placed into boiling water and then gently held against the surface of the skin for about 1.5 seconds. Juvenile sockeye salmon, rainbow trout, fingerling spring chinook salmon, and coho salmon were marked using this "mild heat" procedure. The authors found the brands enlarged as the fish grew but began to fade after a year.

Fujihara and Nakatani (1967) felt that the hot-wire method of Watson (1961) and Johnson and Fields (1959) was unsatisfactory because of the degree of injury suffered and the short-term clarity of the marks. The work of Groves and Novotny (1965) suggested to them that extreme cold or "cyrocautery" could also be used to mark fish. They immersed branding tools similar to those developed by Groves and Novotny into a -80°C slurry of dry ice and ethanol and branded rainbow trout, northern squawfish, large scale sucker, carp, and mountain whitefish. The marks were detectable for about 6 months before fish growth and healing made them undecipherable.

Fujihara and Nakatani (1967) also used the mild-heat method of Groves and Novotny (1965). After comparing the two techniques, they concluded that freeze branding was less traumatic, simpler to use, 'and produced clearer brands because of a smaller chance of slippage at the time of marking.

Everest and Edmundson (1967) also found that the mild-heat method did not consistently produce clear bands when they used it to mark juvenile chinook salmon and steelhead. A key factor was the length of time the brand was held against a fish. If branding lasted longer than 1.5 seconds, the fish were burned and lesions sometimes occurred. When a shorter period of time was tried the marks were faint and unreadable. These investigators then tried brands that had been immersed into a -78°C dry ice/acetone mixture and obtained consistent marks that lasted up to 5 months.

However, Mighell(l969) found that branding tips chilled in dry ice slurries often accumulated ice and mucus, which interfered with the heat-transfer process and caused poor marks. To circumvent this problem he used -196°C liquid nitrogen as a coolant. Additionally, he

developed brands with removable tips that could remain in a coolant reservoir. This allowed the silver tip of each brand to remain constantly chilled. Juvenile coho salmon, sockeye salmon, and steelhead were marked by picking the fish up and lightly pressing them against a chilled tip. The resulting brands were sharply defined and remained identifiable on some fish for up to 14 months.

Since Mighell's (1969) technique was developed, liquid nitrogen with fixed or &movable brands has been used by a considerable number of other investigators (e.g., Piggins 1972, Coutant 1972, Raleigh et al 1973, Smith 1973, Turner et al 1974, Park and Ebel1974, Laird et al 1975, Refstie and Aulstad 1975, Dando and Ling 1980, Nahhas and Jones 1980, Gunnes and Refstie 1980, Sorensen et al. 1983, Knight 1990). Fish have also been branded by using freon (Brock and Farrell 1977) or compressed liquid CO^2 (Bryant and Walkotten 1980, Bryant et al. 1990) as coolants. In the latter case, pressurized liquid CO^2 obtained from a fire extinguisher was regulated through a reinforced line to a silver-tipped brand The resulting unit is portable and the flexible hose allows the continuously chilled brand to be brought to the fish.

However, the use of hot wires to brand fish has not been discontinued. Coombs et al. (1990) used a rheostatically controlled transformer to heat 0.5 mm stainless steel wire. Once the wire became glowing red, it was placed on a fish for less than 1 second. Atlantic salmon parr marked in this manner retained readable marks for 8 months, and some individuals had recognizable marks after 3 years. A modified Whal soldering "iso-tip" connected to a rheostat was used by Joyce and El-Ibiary (1977) to mark small (6.7 to 26.5 g) channel catfish. Marks were clearly discemable for 5 months, and when larger catfish (450 g) were branded they retained marks for at least 1 year. Owens and Gebhardt (1968) marked juvenile striped sea perch, cutthroat trout, rainbow, Atlantic salmon, coho salmon, chinook salmon, and english sole with an electro-desiccating instrument. These brands faded in about 6 months.

Nevertheless, most investigators that have marked fish with brands have used cold techniques. Bryant et al. (1990) state these procedures are the most effective. Marking guidelines issued by the American Society of Ichthyologists and Herpetologists (ASIH) (1988) (as cited by Moring 1990) also recommend freeze branding over heat or electrical branding methods when experiments last longer than several months.

Freeze brands, whether freely portable or firmly attached to coolant reservoirs are typically made of copper and silver. The most common combination is a hollow copper stem with an attached silver tip containing a raised silver symbol. This type of brand was first introduced by Groves and Novotny (1965). Other investigators have used industrial steel (Coutant 1972), brass rods with silver or iron tips (Raleigh et al. 1973, Brock and Farrell 1977) and solid copper (Refstie and Aulstad 1975, Dando and Ling 1980, Knight 1990) or silver (Refstie and Aulstad 1975, Gunnes and Refstie 1980, Bryant and Walkotten 1980) brands. Refstie and Aulstad (1975) showed that solid silver brands had better heat transfer characteristics than those made out of copper. Nichrome heating coils or stainless steel wires are generally used when wires are electrically heated to create brands. A systematic comparison that evaluates brand materials, fish size, and symbol size with mark retention has not been done. Nevertheless, freeze brands made from either silver, copper, or some combination of these metals have been used quite successfully to mark a broad array of fish sizes and species.

Suitability of Branding for the NATURES Project

The ideal visible mark for the NATURES program would be one that is behaviorally and physiologically benign and detectable on free swimming fish that are 1 to 3 meters from an observer. Additionally, these attributes need to last throughout the freshwater residency of a

marked individual. A natural hierarchy of questions is generated by these needs (i.e., What are the physiological impacts of branding?; Are the behavioral interactions of marked fish similar to unmarked individuals?, and What is the visibility and retention of such marks?).

Physiological Effects--Potentially, branding procedures can increase mortality, interfere with growth, and induce infections, depending upon how a marked site heals. Mortality caused by electrical, mild heat, and freeze branding is generally quite iow and comparable to that of control populations (Watson 1961, Groves and Novotny 1965, Fujihara and Nakatani 1967, Mighell1969, Smith 1973, Turner 1974, Joyce and El-Ibiary 1977, Dando and Ling 1980, Bryant and Walkotten 1980, Bryant et al. 1990). In a few instances, unacceptably high rates of mortality have occurred. Three factors appeared to be associated with this mortality: excessive pressure at the time of branding (Nahhas and Jones 1980), prolonged branding times (Refstie and Aulstad 1975), and brands with relatively large surface areas (Refstie and Aulstad 1975).

The effects of branding on growth have not been extensively studied. Joyce and El-Ibiary (1977) examined growth rates of 20- and 30-week-old catfish that been hot branded and found that branding initially decreased growth rates in both age groups. After a year of rearing, 30-week-old fish had recovered and were comparable to control fish, whereas the fish branded at 20-weeks of age failed to fully recover. No comparable studies have been performed on salmonids, although a number of authors have assumed that branding has minimal physiological effects on these fishes (e.g., Coombs et al. 1990).

A comprehensive examination of the effects of liquid nitrogen branding on the integument was conducted by Laird et al. (1975). They found that cold branding created a white layer of frozen mucus and epithelial tissue. This spot thawed rapidly and left a clearly defined dark brand surrounded by a white halo. Melanophores directly under the brand became damaged and were only able to exert maximum black pigmentation, regardless of light conditions or skin hue. The halo was ephemeral and caused by extremely contracted melanophores. As the epidermal tissues under a brand healed, the mark became more diffuse. This phenomenon was linked with the proliferation of abnormal appearing melanin-containing cells in the spongiosum and hypodermis tissues (for more details on the structure of fish skin, see Hawkes 1983) associated with the branding site (Laird et al. 1975).

A similar response typically occurs in salmonid fishes and other teleosts as part of the normal healing process (Roberts et al. 1973a, b, c; Smith 1935, as cited by Laird et al. 1975). In fact, when Laird et al. (1975) examined tissues obtained from Atlantic salmon grilse that had been branded as smolts by Piggins (1972) they found that long-term marks were largely derived from the accumulation of corial melanin-containing cells, similar to those found in healing wounds. Such cells generally disappear from healed areas in about 2 years, and Laird et al. (1975) speculated that brands would also vanish after this period. The histopathological survey conducted by these investigators generally indicated that freeze branding caused no long-term deleterious effects. They also stated the technique can be safely used on sahnonids as small as 5 cm.

Behavioral Effects--No quantitative studies have been performed that compare the behavior of branded and control fish. A few scattered anecdotal observations have been made. For instance, Watson (1961) stated that newly branded sea herring swam vigorously and erratically, sometimes breaking the surface of the water before sounding to join a school. Nahhas and Jones (1980) found that fish that had been branded with excessive pressure swam in circles or in a tilted fashion.

In other cases, newly branded fish were causally observed for periods of time (Groves and Novotny 1965, Mighell 1969, Smith 1973) and no anomalous behavior was observed. Before

branding can be considered as a marking technique in the NATURES program, careful behavioral bioassays will have to be performed. These should examine whether a brand interferes with social interactions, foraging success, and susceptibility to predation.

Visibility and Retention--When examined collectively, the results of branding studies indicate that mark retention and clarity can range from weeks to years. Raymond (1974) stated that brand size, symbol type, area branded, time and pressure applied at branding, degree of scalation, and species differences can affect the longevity of brands. We also believe that the "growth potential" of a fish may influence mark retention. Fish that are marked when little additional growth is likely to occur seem to retain marks for longer periods of time than those that have received marks at an early stage in their life cycle (Mighelll969, Smith 1973).

Usually branding symbol size varies with fish size. For example, when salmonids $\leq$ 50 mm have been marked, symbols ranged from 1.6 mm (Smith 1973) to 5 mm by 1 mm in height (Bryant et al. 1990). Parr or smolting fish have typically been branded with larger symbols (6 to 10 mm in height by 2 to 6 mm in width). Smith (1973) compared the retention and clarity of 3.2 mm and 1.6 mm "V" brands on 32-48 mm chinook salmon, coho salmon, and sockeye salmon fry. The larger V consistently produced a sharper and clearer mark.

Long-term retention of 6.25 mm by 6.25 mm and 4.7 mm by 4.7 'mm brands placed on smolting chinook salmon and steelhead were compared by Park and Ebel(1974). They also felt that larger brands provided a better retention rate. However, care must be taken to determine how symbol size, fish size, species marked, and branding duration interact with each other. Refstie and Aulstad (1975), for instance, found that brown trout marked with a relatively large brand (7.5 mm by 3 mm) experienced a 21% mortality rate. Cohorts marked in the same manner with a 5 mm by 3 mm brand experienced no mortality

Symbol shape can also have a pronounced effect on mark clarity and retention. In general, symbols with open designs like 1, 7, T, V, etc., and with lines not exceeding 1 mm in thickness, produce the best marks (Raleigh et al. 1973). Thick lines or brands with closed designs like 5, 6, 8, 9, and 0 tend to freeze surrounding tissues and become blurred (Raleigh et al. 1973). Additionally, Bryant et al. (1990) suggested that similar shaped symbols be avoided, as they found fish marked with V and U brands difficult to separate.

Joyce and El-Jbiary (1977) used a numerical coding system developed by Moav et al. (1960) to place two-digit symbols on the fish they branded. The code, which consists of the symbols representing the numbers 0 to 9 and has been used by other investigators (e.g., Bryant and Walkotten 1980). In other instances, mark location and shape have been combined to create a coding systems with a large number of individual marks (Refstie and Aulstad 1975, Coombs et al. 1990).

Another factor that affects mark retention is the site branded. Raleigh et al. (1973) hypothesized that finely scaled areas would provide the best branding sites because distortion caused by growth would be minimized. They marked adult trout in four areas: the operculum, just above the pelvic fins, anterior to the pectorals, and on the upper back between the dorsal fin and head.

Good marks were obtained on the operculum, but its uneven surface made it a difficult site to brand The best marks were obtained at the finely scaled pelvic fin and anterior dorsal sites. Groves and Jones (1969) found that marks placed anterior to the dorsal fin often became distorted and suggested marks should be located below and posterior to this fin. However, Atlantic salmon

branded by Piggins (1972) retained brands placed on their upper right shoulders for over a year, even though the fish had grown from smolts to 2 to 3 kg grilse.

Fujihara and Nakatani (1967) also examined the influence of mark sites on retention. They found that in areas with heavy scales, the scales interfered with heat transfer and these areas were thus poor marking locations. Coombs et al. (1990) reported that a lack of contrast made brands placed below the lateral line difficult to see. Moreover, brands placed on the caudal peduncle were less frequently detected than those located under the dorsal fin or between the head and dorsal fin. The above observations suggest that the best marking sites are above the lateral line and adjacent to the dorsal fin. Not surprisingly, this is the area most commonly marked on salmonid fishes.

Two other factors are known to affect mark quality: duration of branding and pressure. Chilled brands are usually applied to the surface of a fish for 2 to 3 seconds. When the brand is lifted off, a white frost mark appears where the brand was applied. Bryant et al. (1990) used this visual cue to determine that a 2-second marking episode was appropriate for their marking technique. Longer exposure times produce blurred marks (Everest and Edmundson 1967, Raleigh et al. 1973, Nahhas and Jones 1980), loss of dermal tissue and open wounds and sores (Raleigh et al. 1973; Refstie and Aulstad 1975). Marks will, however, rapidly fade if brands ate not applied for a long enough period (Mighelll969). Park and Ebel(1974) decreased brand exposure from 1-1.5 seconds to 0.5-l seconds when they increased the surface area of the brands they were using.

Most investigators report that they used firm and even pressure when applying brands. Mighell (1969) stated that a fish should be pressed against a brand until the skin is slightly indented. He also discovered that fish should not be rolled across the surface of a brand, as this interfered with brand quality.

The only quantitative assessment of branding pressure we could find was conducted by Nahhas and Jones (1980). They constructed brands that weighed varying amounts (13.5 g to 95.6 g), chilled them in liquid nitrogen, and let them rest without additional pressure on the surface of a fish. Therefore, a brand that weighed 13.5 g, for example, exerted this amount of pressure at marking. The best marks were produced by brands that weighed between 20 and 28 g. Ideal marking pressures and times are undoubtedly affected by brand materials, symbol size, coolants used, fish size, species, and mark location. Even so, the above studies suggest that 1 to 3 seconds of light pressure should produce serviceable marks in most cases.

Summary and Recommendations for Brand Marking

Previous experiments have shown that various branding techniques can successfully mark juvenile salmonids for up to several years. However, no investigators have evaluated whether branding affects growth, behavior, and maneuverability in juvenile salmon. These assessments are critical for the NATURES program because the efficacy of planned treatments will be determined largely on the basis of behavioral criteria (e.g., on avoidance of predation, foraging success, social behavior, water column positioning, and so forth).

Before such evaluations can occur, a standard method of branding needs to be established. After reviewing the above literature, we recommend that the following branding procedures be used on salmonids when they are $\leq$ 100 mm:

First, the fish should be anaesthetized in MS 222 prior to branding. Second, the coolant and brand-tip arrangement described by Bryant and Walkotten (1980) should be adhered to because it has several important advantages over other branding methods.

For example, compressed CO_2 is used as the coolant in their system rather than liquid nitrogen. This gas is safer and easier to obtain than liquid nitrogen, and portable field-branding units can be assembled since liquid CO_2 is often used in small fire extinguishers. Moreover, because the brand is attached to reinforced hosing it is continuously chilled (-109°C) but still flexible enough to be brought to a targeted area on a fish.

The brand tip should be made of silver since this metal apparently transfers heat more efficiently than copper, is malleable, and is still relatively inexpensive. The literature generally suggests that symbol size should vary with fish size and that juvenile salmonids $\leq$ 50 mm should not be marked with brands exceeding 3.2 mm by 1 mm. Consequently, brands of about this size or smaller should be used when fish are smaller than 100 mm.

Symbol shapes should be defined by lines 0.2 mm to 0.5 mm in thickness. Exactly what these shapes should be is still an open question. In most cases where branding has been used to mark fish, detection has occurred after a fish has been removed from the water. In the NATURES situation, marks that remain visible underwater for at least several months are needed. Previous studies have shown that easily detected shapes consist of open designs that possess at most one angle (Refstie and Aulstad 1975).

However, it is unknown whether relatively large, and starkly artificial, marks are desirable or if shapes that resemble natural marks are more appropriate for NATURES investigations. The determination of suitable symbol shapes is yet another question that needs to be addressed if this marking method is going- to be used in the NATURES program. Finally, branding time and pressure should follow those recommended by Bryant et al. (1990).

Although branding has been used to mark fish for over 40 years, many important assumptions about its impacts on fish remain unanswered. This is not atypical for marking procedures. In general, most marking studies are concerned with whether a technique produces a mark, how long the mark will last, and possibly whether the marking procedure induces mortality or easily seen impairments. Often behavioral and physiological effects are not evaluated and assumed to be minimal or transitory. As stated above, however, the types of information required from marked fish in the NATURES program requires that careful assessments of these effects be made. Thus, we feel an important contribution of the NATURES investigations will be to conduct such appraisals on a variety of marking techniques.

Laser Marking

Historical Development of the 'Laser Marking Method

In the early 1970s, K. Farrell, T. Bell, and colleagues at Washington State University (WSU) performed a series of fish-marking experiments with a ruby laser (Hawkes 1973). Very little of this work was published, but it is clear that coho salmon, steelhead, and catfish were successfully marked (Hawkes 1973, 1976, Raymond 1974, Brock and Farrell 1977). NMFS researchers collaborated with the WSU experimenters and conducted some field trials using laser marked fish. In a review of this work, Raymond (1974) reported that the method was difficult to use because of fluctuating voltages and the inability to produce accurate beams with desired wavelengths..

Nevertheless, long-lasting laser marks were successfully placed on fish. A Konrad laser system with a K-15 power supply, K-l head, Q-switch, and 10-mm ruby lens was used by Brock

and Farrell (1977) to mark 12-to 15-cm catfish. Fish were anesthetized, placed into a container of water and positioned in front of a Pyrex Port. The laser beam was directed through the port and water layer before it struck the fish. Although the duration of the laser pulse was not mentioned, laser power ranged between 4.0-4.6 V (joules/cm^2) and the beam produced circular marks 10 mm in diameter that grew with the fish and were still visible after 1 year.

Hawkes (1973, 1976) used the same laser system to mark juvenile coho salmon. The intent of her studies was to examine the histological effects of laser marking on fish. She did mention, however, that visible marks remained on steelhead for as long as 2 years. Moreover, Hawkes (1976) observed that the duration of a laser pulse (when power was kept constant) could affect mark retention. Coho salmon exposed to a millisecond beam pulse lost their marks in about 1 month, whereas those that were irradiated for 30 nanoseconds retained their marks for 6 to 8 months.

Since this initial work, laser technology has evolved and difficulties such as fluctuating voltage and beam regulation are no longer problems (L. Blankenship, WDFW, Olympia, WA., pers. comm.). Because of these advances, the technique has been recently resurrected by Microoptical Engineering, the company that used a medical laser to mark juvenile coho salmon and fall chinook salmon during the spring of 1992. Some of these fish were transferred to the WDFW George Adams Hatchery, where we had a chance to observe them and evaluate mark visibility and retention (see last section of this appendix for details). Our evaluations indicated that the technique produces highly visible marks with little overt stress. However, many of the marks disappeared after 3 months and it is clear that additional experimentation will have to take place to ascertain beam energy and exposure parameters that will provide better mark retention times.

WDFW recently acquired a laser identical to the one used by Microoptical Engineering and additional laser marking experiments will take place in the near future,

Suitability of Laser Marking for the NATURES Project

Laser marking could be a very useful technique in the NATURES program. As discussed below, this marking method appears to be less traumatic and stressful than branding, and it potentially can be used on very small, even newly emerged salmonids. Additionally, in the laser used by Microoptical Engineering, the light beam is carried by a complex of fiber optics (L. Blankenship, WDFW, Olympia, WA., pers. comm.). Consequently, by shutting off selected fiber channels it is possible to create marks with variable shapes. Besides creating "brand" like marks, we also believe laser beams could be used to scar soft fin rays (e.g., in the dorsal, caudal, and anal fins) and, therefore, create visible marks similar to those reported by Rinne (1976) and Welch and Mills (1981).

Physiological Effects--Even though laser marking has existed for over 20 years, we were unable to find any quantitative assessments on how it may influence post-marking growth or mortality. Investigations performed by Hawkes (1973, 1976) suggest that physiological impacts are probably minor, although like any artificial marking procedure, laser irradiation can potentially affect growth and mortality, and induce deleterious trauma or stress.

Lasers produce a beam of monochromatic light that can be unfocused or focused to a very small (0.1 μm) spot (Hawkes 1976). Unfocused beams have been used to mark fish, and when these are directed at tissues, molecules absorbing the wavelength of the laser may become damaged (Hawkes 1976). Hawkes (1973, 1976) examined the ultrastructural effects of an unfocused laser pulse on the skin of juvenile coho salmon. Anaesthetized fish were marked just below the dorsal

fin by either a 30 nanosecond or 1 millisecond pulse of 6,943 lambda light from a ruby laser. Before striking the targeted area, the laser pulse first went through a quartz filter and then 4 cm of water. Cellular effects were determined by periodically collecting skin samples from treated fish.

A number of color changes occurred in the irradiated area. A transitory blanching developed, which was followed by a gradual darkening and hyper-pigmentation of the target area. After 6 to 8 months, the mark area turned brown and eventually regained its normal coloration (Hawkes 1976). Some histological knowledge of the skin is necessary to understand how such color changes occurred and to assess the potential of creating long-lasting laser marks.

Salmon skin has two main layers: an epidermis which is usually 5 to 10 cells thick in juvenile salmonids, and a thicker dermis which lies between the epidermis and underlying muscle (Hawkes 1983). The dermis has an outer layer called the stratum spongiosum that is composed of bands of collagen, fibroblasts, and pigment cells. Directly beneath it is the stratum compactum, which is largely composed of sheets of collagen and scattered fibroblasts. Below this is a loose connective-tissue layer called the hypodermis, which lies adjacent to the muscles (Hawkes 1983). Hawkes (1976) found that nanosecond laser pulses caused no overt damage to the epidermis; cells were not lost nor did necrosis occur. However, some pigment cells in the dermis were damaged or destroyed by the laser.

Three types of pigment or chromatophores exist in the dermis: iridophores, xanthophores, and melanophores. They can be differentiated from one another by their pigment granules, shapes, and associations, (Lagler et al. 1962, Hyman 1964, Hawkes 1983). Xanthophores contain drosopterin and carotenoid granules, which are yellow and are scattered throughout the dermis. Iridophores are globular shaped and hold the reflecting platlets of guanine that give the fish its silvery appearance. They usually form 2 to 10 cell clusters that arc closely associated with 1 to 3 melanophores. Melanophores are dendritic cells that contain melanin, a substance that absorbs light throughout the entire visible range. Although their cell bodies are located below the iridophores, they send branches upward which cover the reflecting iridophores (Hawkes 1983).

Iridophores were extremely sensitive to laser light, and all of them were destroyed in an irradiated area (Hawkes 1976). Melanophores, on the other hand, were either killed, partially destroyed, or damaged in such a way that they were no longer able to aggregate their melanosomes (small sacs 0.5 μ in diameter that contain melanin). Typically, melanosomes are concentrated in the center of a melanophore, which causes the skin to appear light. The inability to concentrate melanosomes therefore forces an irradiated zone to become black (Hawkes 1973, 1976).

Laser irradiation‘affects melanophores because the melanin contained in the cell absorbs the beam’s energy, and this in turn may cause the melanin to break down or the melanosomes to burst (Hawkes 1976). Dead melanophores are removed by macrophages and replaced by new ones; consequently, laser marks can quickly disappear if beam strength or duration kills most of the melanophores in a marked area.. Thus, to create durable laser marks, one must ascertain how much energy can be absorbed by melanophores to establish long-living patches of disabled cells.

Xanthophores, along with other cells in the dermis are not affected by laser beams (Hawkes 1976). When these cellular effects are compared to those described by Laird et al. (1975), one gets the impression that laser marking creates less trauma to the skin than freeze branding. Moreover, fish can be marked without removing them from water and this probably is also less stressful than the methods typically used to brand fish.

Another potential way to mark salmonid fishes with laser beams is to either selectively remove or create scars on their soft fin rays. In the past, marks of this type have been produced by using small surgical scissors to completely cut through a spine or soft ray. Rinne (1976) marked three species of Talapia by cutting the most proximal end of their spines, leaving the interconnecting membrane between spines intact. By removing selected spines in the dorsal and anal fins, a considerable number of individual marks could be produced. Spine regeneration, however, was relatively high in small fish (5-9 cm), and marked individuals were often difficult to identify several months after marking.

Rather than cutting the proximal ends of spines, Welch and Mills (198 1) cut soft rays some distance from their point of attachment, Like Rinne (1976), these investigators left the membrane separating the soft rays intact and did not cut more than two adjacent rays, since this caused the distal ends of the cut rays to be sloughed off. Within a month, prominent round scars occurred in the cut rays. The smallest fish that Welch and Mills (1981) marked were 12-cm arctic char, lake white fish, and lake trout. They found no evidence of mark loss in wild fish but marks were lost in hatchery fish because of the fin deformities that often occur in crowded raceways and ponds.

We concluded that a focused laser beam of the type used in microsurgery could be used to quickly cut soft rays in salmonids. It is possible that such cuts would heal differently than those produced by surgical bone nippers, since both ends of the ray would likely be cauterized by the beam. Whether this would interfere with the healing process is unknown, but if the round scarring phenomenon did manifest itself, good visible marks could be produced on very small fish.

Rinne (1976) and Welch and Mills (1981) cut rays in the dorsal and anal fins, yet both are often folded down in swimming fish and therefore hidden from view. When observing the underwater visibility of marks on free swimming and caged salmonids, we noted that the caudal fin was usually fully opened. Consequently, the rays in this fin may be good marking sites, particularly if only one or two rays are cut. Another important advantage associated with fin ray marks is that they are visible on both sides of a fish; this attribute clearly facilitates rapid recognition by observers.

Growth, mortality, and trauma effects caused by spine removal or soft-ray scarring have not been formally examined. Both Rinne (1976) and Welch and Mills (198 1) felt that the procedure had a minimal impact on marked fish although no special studies were undertaken to confirm this.

Behavioral Effects--No studies have been conducted which compare the behavior of unmarked control fish with those receiving "brand-like" laser marks. Additionally, the behavioral effects of selectively cutting soft rays or removing spines in fins has not been evaluated. If laser marking is to be used in the NATURES program, such investigations must be performed.

Visibility and Retention--Our experience with laser marks indicates that they can be highly visible underwater, and that their distinctness is affected by where they have been placed and by the beam parameters used to create them (as discussed in the preceding section). In three instances the long-term retention of such marks has been evaluated (Hawkes 1973, Brock and Farrell 1977). It has ranged from 6 to 22 months in salmonids and for at least a year in catfish.

Like brands, laser marks placed on the integument will fade and enlarge with fish growth. On the other hand, if soft-fin ray scarring can be induced by laser cutting, these marks may last throughout an individual's lifetime. In general, there is very sparse literature on laser marking, and with the advent of new types of lasers and fiber-optic beam control we simply cannot evaluate

mark retention or visibility. Consequently, this is an area, like behavioral effects, that must be examined in the future.

Summary and Recommendations for Laser Marking

The above sections should make it clear that some basic mark effect, visibility and retention work needs to be accomplished before laser marking can be implemented in the NATURES study. We believe, however, that the technique holds great promise and may be more benign than branding. Additionally, the possibility that focused laser beams can be used to create visually detectable scars in soft fin rays should be examined.

Subcutaneous and Visual Implant (V.I.) Tags

Historical Development of Methods for Subcutaneous and Visual Implant Tagging

Subcutaneous or visual implant tags for fishes have been developed at least three times over the past 15 years. Such tags were first created by Heugel et al. (1977) who employed them to tag small (≥ 16 mm) fishes while investigating the population dynamics of several Poecilid species.

Their tags were produced by placing four-digit codes in columns and rows on a 25 by 36 cm sheet of white paper. The paper was then photographed and 1 by 3 mm tags were cut from a resulting diazo contact print To facilitate insertion and enhance retention, the tags were shaped like arrow heads. Fish were tagged by making a small incision in the musculature, just anterior of the dorsal fin, and microforceps were then used to completely imbed the tag under the skin.

Inserted tags were visible for up to 6 months, although by that time, melanophores had often proliferated enough to completely obscure the tag. However, the melanophore patch was used to indicate that a tag was present on a fish and Heugel et al. (1977) were able to hold fish with such markings for at least 18 months. In a number of instances, tags that had been covered by melanophores were removed to determine if their four-digit codes were still legible: in all cases the codes could be clearly seen.

The major disadvantages of this method are that losses will be high if the tag is not completely embedded in the musculature and that the tagging process is relatively slow (Heugel et al. 1977). Moreover; Joswiak et al. (1978) found that the tags were uniformly rejected by fishes in the Cyprinidae family. Tag rejection was characterized as a 3- to 4-day phenomenon that followed the same pattern as wound healing.

Generally, acute inflammation rapidly occurred around the tagging lesion, followed by the destruction of adjacent muscle and epithelial tissues, which lead to the ejection of the tag (Joswiak et al. 1978). Fishes representing the Poecliidae, Cichlidae, Centrarchidae, and Percidae families were also tagged but did not exhibit the rejection response. Salmonid fishes were not tested by Joswiak et al. (1978) but as will be discussed below, it is likely that they too, would not reject such tags.

Shortly after the advent of the above diazo tags, the U.S. Fish and Wildlife (Anonymous 1980) tried marking fish with Microtaggants, which were small pieces of laminated plastic material manufactured by the 3-M Company. Microtaggants were injected into the fins, under scales, by the gular, opercular, and other body-wall areas of blue gills, carp, striped bass, and catfish. Injections were delivered by syringes, air brushes, high-pressure water, and high-pressure air

systems (Anonymous 1980, Zak 1984, Thompson et al. 1986, Klar and Parker 1986). The best delivery system proved to be a needle-less hypodermic injector with a modified nozzle.

Maximum retention equaled 99% after 6 months, and in some cases marks that were not visible under normal light conditions were detected 2 years after marking by using an ultraviolet light source to excite fluorescent materials present in the tags (Thompson et al. 1986). As mentioned at the onset of this review, the capacity to create invisible marks that can be revealed by remote interrogation is an important mark or tag attribute for the NATURES program.

Whether Microtaggants could be used to create such marks is a moot question, since they arc no longer manufactured However, alternative sources of small fluorescing spheres are available (e.g., the Duke Scientific Corporation), and we believe they could be incorporated into V.I. tags. Ideally such tags would be invisible to fish and human observers unless activated by a source of ultraviolet light.

Beginning in the late 1980s, Northwest Marine Technology (NMT) and WDFW researchers started to develop and test three additional V.I. tags (Haw et al. 1990, Bergman et al. 1992). The genesis for this work was the need to develop tags with alphanumeric codes that could be visually detected on live fish. Initially attempts were made to create small external tags using biocompatible materials; however, because of the percutaneous attachment problem (Buckley and Blankenship 1990, Haw et al. 1990, Bergman et al. 1992) this effort was abandoned, and shallow subcutaneous injection of small tags was tried on a number of species.

For instance, full-sized (1 by 0.25 mm) coded-wire tags (CWTs) were injected above the eyes and parallel to the long axis of the body in 35-72 mm pumpkinseeds; surprisingly these tags remained clearly visible for 19 months (Haw et al. 1990). The same technique was tried on 42-70 mm slenderhead darters, but scalp tissues were too thin, so CWTs were injected into pre-opercular tissue. CWTs proved to be too large, and they eventually eroded out; however, when monofilament polyproplyene suture material was used, the tags were retained and remained visible for 13 months.

When this technique was tried on juvenile salmonids, it was discovered that these fishes possess a clear adipose "eyelid" that lies posterior to the eye (Haw et al. 1990). This postorbital adipose tissue is commonly transparent, relatively acellular, contains little fat, and few vessels, and is generally similar to corneal tissue (Haw et al. 1990; L. Blankenship, WDFW, Olympia, WA, pers. comm.).

Altogether, the NMT/WDFW investigators have inserted four different types of tags under the eyelid: the aforementioned CWTs, and three new types. One of the new tags is made from a material called elastomer, which is a biocompatible rubber-like substance, impregnated with fluorescing particles. A simple syringe can be used to inject elastomer, but more sophisticated applicators that provide uniform lengths of the material are available from NMT.

Just prior to use, an elastomer base and a catalyst are mixed with a hardener and loaded into an applicator. This soft mixture is then inserted under the postorbital adipose tissue where it hardens into an about 1.0 by 0.25mm band of colored material (P. Bergman, NMT, pers. comm.). At our request, NMT produced a clear-appearing elastomer that fluoresced blue when exited by UV light. It should also be possible to produce other clear-appearing elastomers with different fluorescing colors. If such marks can be excited underwater and are detectable by observers 1 to 3 m away, elastomer bands could become an important evaluation tool in the NATURES program.

A second new tag type, made from stiffened suture material, was also tried. Like the elastomer tags, these were colored, and hence, with some customizing it should be possible to produce fluorescing suture material that is nearly invisible under normal light conditions. These tags were also approximately 1.0 by 0.25 mm and were fashioned in this way so that a continuous reel of suture "wire" could be used in a standardized CWT tagging applicator (P. Bergman, NMT, pers. comm.).

The third new type of V.I. tag tried was a flat mylar wafer or diazo film tag (Haw et al. 1990). Unlike the elastomer and suture tags, alphanumeric information can be encoded into the tag and therefore individual fish recognition is possible. Generally, these tags have ranged from 0.5-1.5 mm wide by 1.5 to 4.0 mm long by 0.05 to 0.08 mm thick (Bergman et al. 1992). The shape, size, and material composition of the alphanumeric tags has not yet been established, but research to determine optimal tag attributes will take place in the future (P. Bergman, NMT, pers. comm.).

Suitability of Visual Implant Tags for the Natures Project

As suggested above, V.I. tagging could be a very useful technique in the NATURES program. We had an opportunity to routinely observe juvenile fall chinook salmon (> 70 mm) that were tagged with brightly colored fluorescing elastomer in our viewing chamber. In general, marked fish were accurately identified when they were 1.5 to 2.0 m from a viewing window. V.I. tags made from suture material may prove to be equally conspicuous, but we did not assess their visibility.

Flat, alphanumeric V.I. tags may not be as effective as the elastomer types because the coded letters and numbers are small and may be difficult to decipher in fish that arc 1 m or more away from an observer. Creating solidly colored, flat V.I. tags is a possibility, although (as we discuss) the retention rate of these tags can be low when used on small salmonids (I 125 mm).

The major constraint associated with currently extant V.I. tags is that salmonids must be relatively large (≥ 70 mm) to achieve acceptable retention rates. Even given this limitation, we can see many opportunities in the NATURES evaluation effort where such tags could be effectively utilized if they have a neutral effect on behavior and physiology.

Physiological Effects--Haw et al. (1990) observed the reaction of postorbital adipose tissues to flat, laminated V.I. tags. It ranged from none to the formation of a thin fibrohistiocytic envelope around the tag, which was accompanied by a mild chronic inflammatory infiltrate. Our impression was that when a tissue response was noticed, it was probably caused by the glue used to create the layered tags.

Observations of reactions of the adipose eyelid to elastomer, suture, and CWTs have not to our knowledge been made. However, in the first two cases tags are made out of biocompatible materials, so it is quite likely that they will have an even more benign effect on fish than that described for the flat tags. No growth or mortality assessments have been made on these tags, although rainbow trout that had CWTs inserted into their postorbital adipose tissues had virtually no mortality for over 137 days (Anonymous 1990, 1991).

Behavioral Effects--No quantitative studies have been made that compare the behavior of fish possessing V.I. tags with non-tagged cohorts. On a number of occasions, we casually observed fall chinook salmon juveniles that had been tagged with brightly colored elastomer. While making these observations we did not notice fish nipping at marks or chasing marked fish. If clear V.I. tags that fluoresce different colors can be produced, deleterious behavioral effects

should be minimized. To test these ideas, however, detailed behavioral assays need to be performed on fish possessing various V.I. tags.

Visibility and Retention-Kincaid and Calkins (1992) conducted a study which examined-the visibility and retention of flat, alphanumeric V.I. tags in juvenile and adult Atlantic salmon and lake trout. Adult fish received 2 by 4 mm tags while 1 by 3 mm tags were used in juvenile fish. Both species and size differences in tag retention and visibility were observed. Generally, Atlantic salmon retained their tags at higher rates than lake trout. For instance, 294 days after tagging 84% of the adult Atlantic salmon possessed tags but only 45% of the lake trout retained their tags.

Differences between juvenile Atlantic salmon and lake trout were not as great (49% vs. 41%) but the visibility of retained tags was noticeably different. Virtually no tags were readable in lake trout juveniles because of increased pigmentation in the postorbital adipose tissue, while 100% of the tags retained in juvenile Atlantic salmon were decipherable. Tag loss was also linked to body size in Atlantic salmon juveniles: every fish that was ≤ 20 g at tagging lost its tag within 70 days, and losses equaled 54% in juveniles that were 21-42 g and 29% in those that were 41-99 g at tagging (Kincaid and Calkins 1992).

During the first 70 days after tagging, tags were lost primarily through the tag wound. After that period, tag edges and comers often eroded and ruptured the overlying adipose tissue and were subsequently shed (Kincaid and Calkins 1992). These investigators concluded that even the small (1 by 3 mm) V.I. tags they used were commonly too large for the amount of adipose tissue available in juvenile lake trout and Atlantic salmon. They also observed some tags migrating downward into dermal tissues, which made the tags difficult to read. In one instance, half of a tag was embedded into the dermal tissues at the time of recovery (Kincaid and Calkins 1992).

This study and other unpublished observations on steelhead trout (Anonymous 1991) suggest that retention of presently designed, flat V.I. tags in small salmonids is not high. Comparable work has not been performed on elastomer and suture tags, but some retention evaluations have occurred on salmonids which have had 1.0 by 0.25 mm CWTs injected into their postorbital tissue (Anonymous 1990, 1991). In these studies, juvenile chinook (91- 181 mm, mean 143 mm), coho salmon (86-136 mm, mean 105 mm), rainbow trout (116-140 mm), and steelhead (122-175 mm) all had high retention rates (96-98%) for up to 137 days. Because suture and elastomer V.I. tags have-shapes similar to CWTs, their retention may also be high.

Summary and Recommendations for Visual Implant Tags

Of the V.I. tags reviewed above, the most promising appear to be those made of elastomer and stiffened suture material. The behavioral and physiological effects (growth, mortality, and tissue reaction) of these tags, as well as their underwater visibility, need to be carefully appraised. Additionally, we feel that research should be directed toward producing clear-appearing elastomer and suture V.I. tags that fluoresce with distinctive colors when interrogated by a UV-light source. The main disadvantage associated with V.I. tags is that they are often shed when relatively small fish are tagged.

In the past, V.I. tags have been single units that are at least 1 mm long. However, use of postorbital adipose tissue as a tagging site by Haw et al. (1990) suggested to us that multiple small taggants could be injected under this tissue. Fluorescing red, green, or blue spheres with diameters ranging from 0.024-2.87 µm are commercially available. It may be possible to produce long-lived fluorescing marks on very small salmon by injecting an isotonic slurry of such spheres

under the adipose eyelid. In addition, more diminutive elastomer and suture tags than presently exist should be tried and evaluated because they may be accommodated by salmonids ≤ 70 mm.

In general, we are optimistic that even at their present stage of development, V.I. tags can be successfully used to help evaluate various NATURES treatments. Yet, as emphasized throughout this review, basic evaluations on the effects of these tags should be carried out to prevent any inadvertent biases in subsequent data analyses.

Panjet Marking

Historical Development of the Panjet Marking Method

Crustaceans, fishes, and amphibians have been marked with stains and dyes for nearly 60 years (Costello 1959). Commonly, these marks have been created by using metal oxides and sulfides, biological stains, acrylic polymer emulsions, fluorescent pigments, liquid latex, cationic dyes, and various forms of carbon (e.g., graphite and India ink). Their retention has ranged from days to years and is influenced by the type of dye or stain used, species being marked, specimen size, body area utilized, and the marking method employed (Table 1).

For instance, direct immersion, hyper-osmotic baths, and daubing of pigments onto the skin rarely produce visible marks that last longer than several weeks. Conversely, hypodermic needles and tattooing devices which inject dyes into the dermis, or tissues that lie just beneath the skin, often generated long-lived marks that were well-defined and could be placed in specific body areas.

Fish have also been successfully marked by using high air pressure to embed dye particles over the entire body surface (Jackson 1959, Phinney et al. 1967, Andrews 1972, Phinney and Mathews 1973, and many others). This technique allows many individuals to be marked at once and prevents disease problems caused by contaminated needles. However, precise control of mark location is lost, and fish exposed to the same marking episode retain varying amounts of pigment.

The advantages of using high air pressure to deliver dyes can be coupled with the ability to place marks in specific body locations if needle-less hypodermic inoculators are used. Kelly (1967) was the first investigator to mark fish with this tool (Press-O-Jet, Z & W Corp.), and subsequent investigators have used Ped-O-Jets (Vemitron Medical Products, Inc.; Klar and Parker 1986), Panjets (F. H. Wright Dental Manufacturing Co, Hart and Pitcher 1969, Starkie 1975, and others), Syrijets (Mizzy, Inc.; Laufle et al. 1990), and Madajets (Mada Medical Products, Coombs et al. 1990).

All of these hand held tools, collectively referred to as panjets, inject materials subcutaneously into relatively small (usually 1 to 5 mm) areas using high air pressure. Typically they possess a glass reservoir which holds marking materials, a hand operated spring plunger to generate air pressure, and a restricted nozzle to aim the pigment.

Like hypodermic needles and tattooing tools, panjets can only mark one fish at a time. Yet high rates of tagging (30 fish per/min) are possible because the technique does not demand that an operator insert a needle and inject dye (Hart and Pitcher 1969, Starkie 1975). Instead, a button or trigger is activated, and the marking materials are then instantaneously delivered via air pressure into a targeted area. During a panjetting episode, fish are usually anesthetized and laid on a sponge saturated with anaesthetic (Hart and Pitcher 1969), the device is then either gently placed on the

Table 1. Maximal retention times of various stains and dyes used to mark crustaceans, fishes, and amphibians.

Dye/Authority	Markingmethod	Maximum retention	organisms marked
Metal Oxides			
Dustsn & Bostick 1956	Tattoo	3+[a] months	Juvenile chinook and coho
Kelly 1967	Hypodermic injection	1 year	Brown trout
Hill et al. 1970	Hypodermic injection	7 months	Channel catfish
Metal Sulfides			
Wigley 1952	Hypodermic injection	1.5+ years	Lamprey ammocoetes
Hansen & Staufer 1964	Hypodermic injection	4+ years	Lamprey ammowetes
Hill et al. 1970	Hypodermic injection	7+ months	Channel catfish
Carbon and Inks			
Wigley 1952	Hypodermic injection	1.5+ years	Lamprey ammocoetes
O'Grady & Hoy 1972	Hypodermic injection	17 days	Mosquito fish
Engstrom-Heg & Loeb 1974	Hypodermic injection	l-2 years	Brown trout
Starkie 1975	Panjet[b]	2-3 months	Dace and brown trout
Cane 1981	Panjet	24 days	Rainbow trout
Laufle et al 1990	Panjet	10+ days	Arctic char and ciscos
Biological Stains			
Dustan & Bostick 1956	Tattoo	87 days	Juvenile chinook and coho
Costello 1959	Hypodermic injection	73+ days	Pink shrimp
Loeb 1962	Feeding	2+ months	carp and trout
Kilma 1965	Hypodermic injection	276+ days	Misc. shrimp species
Lawler & Fitz-Earle 1968	Immersion	7 days	Trout-perch
Hart & Pitcher 1969	Panjet	14+ months	Thirteen species of fish
O'Grady & Hoy 1972	Immersion	17 days	Mosquito fish
Jessop 1973	Immersion	8 days	Alewife fry
Guttman & Creasey 1973	Immersion	10 days	Tadpoles
Starkie 1975	Panjet	18+ months	Dace and brown trout
Pitcher & Kennedy 1977	Panjet	3.5+ years	Roach
Johnstone 1981	Panjet	2+ years	Atlantic salmon
Cane 1981	Panjet	3+ months	Rainbow trout
Coombs et al. 1990	Panjet	6+ months	Atlantic salmon
Axford (pers. comm.)	Panjet	16+ years	English barbel
Liquid Latex			
'Riley 1966	Hypodermic injection	2+ years	Plaice
Hill et al. 1970	Hypodermic injection	7+ months	Channel catfish
Fluorescent Pigments			
Kihna 1965	Hypodermic injection	276+ days	Shrimp species
Duncan & Donaldson 1968	Tattoo	29 months	Juvenile coho
smith 1970	Hypodermic injection	11+ months	Brook stickleback
Ireland 1973	Hot probe laden with pigment	150+ days	Larval salamanders
Imler 1974	Hypodermic injection	3+ years	Walleye
Ryan 1975	Panjet	16+ months	Short finned eel

Table 1. Continued.

Dye/Authority	Marking method	Maximum retention	Organisms marked
Refstie & Aulstad 1975	Tattoo	13+ months	Atlantic salmon
Thompson et al. 1986	Panjet	2+ years	Yazoo darters and striped bass
Pauley & Troutt 1988	Hyperosmotic bath	15 days	Juvenile steelhead
Pauiey & Troutt 1988	Pressurized air	90+ days	Juvenile steelhead
Pauiey & Troutt 1988	Painting with an artist's brush	41 days	Juvenile steelhead
Acrylic Polymer Emulsions			
Kelly 1967	Hypodermic injection	6 months	Brown trout
Hill et al. 1970	Hypodermic injection	28 days	Channel catfish
Benda 1971	Hypodermic injection	8-12 months	Seven species of fish
Wooiiey 1973	Hypodermic injection	19+ months	Two salamander species
Lotrich & Meredith 1974	Hypodermic injection	16 months	Ten species of fish
Cecil & Just 1978	Hypodermic injection	10+ months	Tadpoles
Cationic Dyes			
Kelly 1967	Hypodermic injection	2+ years	Brown trout
Loeb & Kelly 1969	Hypodermic injection	8+ months	Brown trout

a + following the retention time indicates that the experiment ended before the mark disappeared

b A panjet is a hand held, needleless, hypodermic inoculator that uses a spring plunger to create a high-pressure jet spray.

surface of the skin or held 2-30 mm away from the fish at the time of mark application (Starkie 1975, Klar and Parker 1986, Laufle et al. 1990).

The first marking substances used in panjets were biological stains and Indian inks (Hart and Pitcher 1969, Starkie 1975, Pitcher and Kennedy 1977). These highly soluble materials were chosen because dye particles can clog panjet nozzles (Johnstone 198 1). However, if care is taken, granulated materials like fluorescent pigments (Ryan 1975, Thompson et al. 1986) and Microtaggants can also be successfully injected into fish (Klar and Parker 1986).

Finally, because air pressure is being used instead of needles, panjets can mark fins as well as other body areas (Ryan 1975). Because of their versatility and history of creating long-lived marks, we feel that panjets represent the best method of delivering stains and dyes into small salmonid fishes.

Suitability of Panjet Marking for the NATURES Project

As suggested above, one of the key advantages of panjet marking is that small fish (about 40 mm, Thompson et al. 1986) can be marked However, such individuals may be damaged by the procedure if marks are not placed over flat bones or in fins (Johnstone 1981). When applied in appropriate locations, such as fins (Starkie 1975), bases of fins (Hart and Pitcher 1969), operculum or "check patch" (Kelly 1967), or pelvic and pectoral girdles (Cane 1981), the procedure appears to induce a minimum amount of stress (Coombes et al. 1990). Moreover, because both soluble and granulated pigments work in panjets, a variety of different appearing marks can be produced.

Perhaps most importantly, fluorescing materials that produce marks invisible until excited by W wavelengths have been created by panjets (Ryan 1975, Thompson et al. 1986). As previously mentioned, such marks may prove to be very valuable in evaluating how various NATURES treatments influence artificially cultured salmonids. Given these attributes, it seems to us that panjets could become an important marking tool in the NATURES evaluation program.

Physiological Effects--Like the other methods reviewed above, few formal appraisals have been conducted on the effects of panjetting on growth, mortality, and histological effects. The technique can impact fish in two ways: by the trauma of the injection process, and by the interaction of injected materials with body tissues.

The process itself appears to be benign: in the only case where a careful mortality study was conducted, Thompson et al. (1986) found that striped bass (96-198 mm) and yazoo darters (39-47 mm) survived as well as controls throughout l-year (striped bass) and 2-year (darters) holding periods. In other instances, no overt signs of stress or mortality were linked to fish marked with panjets (Pitcher and Kennedy 1977, Laufle et al. 1990). Moreover, Pitcher and Kennedy (1977) observed that body areas receiving panjet marks did not exhibit gross thickening, reddening, or necrosis and that the inflammatory response was limited and localized.

The fate of panjetted materials after injection has only been examined in fins. Pitcher and Kennedy (1977) found that Alcian Blue (a biological stain) panjetted into the lumen of fin rays was neither degraded nor transported from the fin. Apparently the poor blood supply provided to fin rays sequestered the pigments from metabolic processes they probably would be exposed to if placed in other body areas (Pitcher and Kennedy 1977).

A number of other investigators (Kelly 1967; Loeb and Kelly 1969, Engstrom-Heg and Loeb 1974) have examined how tissues marked by hypodermic needles respond to dyes and stains. These studies showed that tissue response varied with the type of marking material used.

For example, when crystalline fragments of dye are injected, they often clump together and line the lumen left by the hypodermic needle. None are ever incorporated inuacellularly into connective tissues, fibroblasts or other cells (Kelly 1967). Fish growth does not affect the shape or size of dye crystals, but they are eventually covered by superficial skin layers which cause the marks to slowly fade (Kelly 1967).

When crystalline dyes are injected close to bones, some particles become confined in the periosteum (the connective tissue that covers growing bone). Such particles are usually transported away from the bone area by the lymphatic vessels associated with the periosteum, and this process can also cause a mark to eventually disappear (Engstrom-Heg and Loeb 1974).

Like granulated dyes, soluble ones are always located extracellularly, but they do not form a concentrated spot around the injection site. Instead, connective tissues and the periosteum of adjacent bones are stained. After about 6 months, the periosteum loses its stain and the remaining dye is closely bound to connective tissue fibers and fibroblasts (Kelly 1967).

These general tissue responses suggest that crystalline marking materials (e.g., metal oxides, carbon, and fluorescent resin particles) can be used throughout the body, but that soluble dyes (e.g., biological stains) should be confined to fin locations. This is particularly important since some soluble dyes have a tendency to rapidly migrate from a marked area (Loeb and Kelly 1969).

We were unable to find any study that compared the growth of control fish with those marked with panjets or hypodermic needles. Travis (1981) examined the effects of immersing tadpoles in Neutral Red (a biological stain) on their subsequent growth and found significant, deleterious impacts. Whether the injection of inert materials into fins or other body areas on juvenile salmonids would have a comparable negative effect is unknown. Our guess is that it probably would not because the work of Kelly (1967), Loeb and Kelly (1969) and Pitcher and Kennedy (1977) has shown that materials injected into fish are often isolated from the rest of the body.

Nevertheless, some simple studies that compare the mortality and growth of control fish with those panjetted with various dyes and stains should be conducted The substances we feel should be tested are Alcain Blue, graphite, and inert fluorescent particles (e.g., Day Glo pigments and the microspheres from Duke Scientific). These materials either have a proven history of producing long-lived marks or could potentially produce marks that are revealed when interrogated by a UV light source.

To further reduce the likelihood of impacts and accentuate underwater visibility, we recommendthatthemarksbeplacedonthecaudalfin.

Behavioral Effects--No quantitative studies have been performed that compare the behavior of unmarked fish with those that have received panjet marks. Thompson et al. (1986) felt that panjetting did not influence the mortality, growth, or behavior of marked fish. A few scattered anecdotal observations have also been made on individuals that have been marked by hypodermic injection.

Smith (1970), for instance, injected small quantities of fluorescent dye under the skin or bony plates of sticklebacks. He observed that marked fish behaved in a normal fashion and that conspecfics were not influenced by marks. Woolley (1973) investigated the effects of injected acrylic polymer on the behavior of cave-dwelling salamanders. Food selection, temperature preferences, substrate selection, and phototrophic responses were examined, and no differences were found between marked and unmarked individuals.

These casual observations suggest that marks produced by panjets could potentially have a neutral effect on behavior. How predators may respond to fish possessing dye or stain marks is however, another matter. We were unable to find any study that evaluated this impact. Plainly, before this technique can be used in the NATURES program, careful studies that compare the behavior of marked and unmarked individuals will have to be conducted.

Visibility and Retention--Indian inks, biological stains (mainly Alcian Blue), and fluorescent pigments have been used in panjets. Mark retention has varied: those made with Indian inks have often disappeared after several months (Starkie 1975; Cane 1981; K. Koski, NMFS, Auke Bay, AK, pers. comm.), while marks made with Alcian Blue and fluorescent pigments have commonly lasted for several years or more (Table 1).

A quantitative assessment of mark retention was conducted by Pitcher and Kennedy (1977) who used a panjet to inject Alcain Blue dye into the fins (upper and lower lobe of the caudal, right and left pectorals and pelvics, anal, and dorsal) of roach. The fish were held for 40 months in a quasi-wild pond and examined at least 32 times. No loss of marks occurred during the first 2 years; however there was a tendency for marks placed on the pelvic fins to be less clear than the other locations. Moreover, when mark loss did occur it was not random, marks which were considered very clear were rarely lost. Pitcher and Kennedy (1977) indicated that if a panjet mark survives for several years it will likely be retained for many further years.

As indicated above, they also found that the best marks were achieved when the dye suspension penetrated into the lumen of a fin ray. Unlike, laser marks or brands, panjet marks placed into fin rays did not change greatly in size. Some color fading occurred and was probably caused by loss of dye from the dermal and connective tissues that surround the fin rays and by chemical changes occurring to the dye itself (Pitcher and Kennedy 1977).

Summary and Recommendations for Panjet Marking

Of all the marking methods we reviewed (brands, laser marks, V.I. tags, and panjetting), we found marks created by panjets had the longest documented retention times. This method can also be used to mark 30-40 mm salmonid fry. Yet, like the other marking tools we reviewed, assessments on the underwater visibility of panjet marks and their effects on behavior, growth, mortality, and susceptibility to predation will have to be made before the procedure can be utilized in the NATURES program.

1992 Field Evaluations to Assess the Underwater Visibility of Select Marks

Introduction

Our review indicated that almost no effort has been directed toward assessing the underwater visibility of artificial marks or how they may influence behavior. During the summer and fall of 1992, we began thinking about how the underwater visibility of different kinds of

marks could be evaluated in a quantitative fashion. At the same time, we wanted to establish observational protocols that could be used to determine whether marked fish 1) behaved like unmarked controls, and 2) were preferentially selected by predators.

We have not yet finalized how these behavioral assays should be performed. Nevertheless, the general approach will be to compare predator avoidance, social interactions, and the foraging skills of marked and unmarked fish. For instance, simultaneous appraisals of social status and foraging will be conducted by observing the frequencies of various behaviors over a standardized time period.

Specifically, assessments of 1) agonistic behavior; 2) body areas attacked (i.e., are marked areas more likely to receive nips than non-marked sites); 3) number of strikes at food/unit of time, 4) water column position relative to cover and depth; 5) coughing, yawning, spitting, and respiratory rates; and 6) number of position changes/unit of time, will be made. These observations will occur in two different types of environments: one that contains complex habitat and one that resembles a standard hatchery raceway.

As is typical for'such studies, 20 to 30 fish receiving the same type of mark will be observed in each rearing area. Predator responses to marked fish will be evaluated by simultaneously releasing smolting fall chinook juveniles with various marks into a stream and later recapturing them at a weir to determine their relative survival rates.

More progress was made with determining how the underwater visibility of marks should be assessed. Two methods were tried, and both were refined by manipulating fish in a custom-made viewing chamber that was filled with water. The remaining part of this review will 1) briefly characterize the viewing chamber, 2) describe the types of visible marks evaluated, 3) recount the methods used to perform the visibility tests, and 4) report the preliminary results obtained from these assays.

Materials and Methods

Viewing Chamber--A 3.0-m-long by 1.2-m-wide by 0.9-m-high fiberglass box with a balsa wood core was built to accommodate our underwater visibility tests and behavioral assays. The box has eight windows, with three (45 cm wide by 70 cm high) evenly spaced on each side and one (60 cm wide by 70 cm high) window is located at each end.

Four of the windows were made with 18.75-cm-thick, tempered glass and the other four were made with 18.75-cm-thick acrylic Plexiglas@. The different window materials were used to assess durability, leakage, and UV transmittance. We found that some leakage did occur around the bottom of the Plexiglas windows and one of the glass windows developed a hairline crack.

Because the glass was laminated with vinyl layers, no UV light could be transmitted through it. Plexiglas II UVT and Plexiglas G UVT on the other hand, transmit all ultraviolet radiation above 275 nanometers. When we placed fluorescing materials in back of the Plexiglas windows and directed W light through it, the materials in the chamber fluoresced, even when the object was totally immersed in water and 3 m away from the light source. Based on this experience, we feel that Plexiglas windows are more versatile and durable than glass ones and consequently recommend that this material be used if additional viewing boxes arc built.

Each end of the chamber was equipped with a flange which the ends were bolted to. The box was designed this way so that multiple units could be connected to one another to create

artificial hatchery raceway environments or stream-like habitats. A 15 cm drain was placed flush with the bottom at each end, and these drains were installed so that large quantities of water could be moved through the box if desired. Water levels can be regulated by an externally located standpipe attached to one of the drains.

To support the box, two 10-cm by 15-cm wooden skids were bolted to the bottom of the chamber, and a cross brace was also attached at the top midway along its length. When empty, the viewing chamber weighs about 230 kg and can be carried by four to six people. It is small enough to be loaded into a full-sized pickup truck and thus can be transported to field locations.

Five sheets of 5.0-cm-thick, tinted Plexiglas that occluded various amounts (10 to 50%) of transmitted light were sometimes used to cover the top of the box, creating two different lighting conditions while the underwater visibility of different types of marks were evaluated. The interior of the box was painted a light brown, but this color created a lot of reflected light and we would recommend a darker shade be used in the future, particularly if chambers are used to mimic various freshwater habitats.

To decrease light entrance into the box from the side windows, we built portable, black polyethylene covers that completely surrounded the box but allowed viewers free access to the windows. Finally, 'to facilitate our visibility assessments, the bottom of the box was marked with lines at 0.25-m intervals from one end of the chamber to the other. These lines were used to help position small Plexiglas boxes that held marked fish.

Types of Visible Marks Evaluated--The underwater visibility of laser marks, adipose fin clips, and elastomer V.I. tags was evaluated in the viewing chamber. On 20 June 1992,199 juvenile fall chinook salmon and 192 coho salmon, ranging in size from 70 to 110 mm were transported from the WDFW George Adams Hatchery (Mason County, Washington State) to Portland, Oregon, where they were marked with a Linear Flashlamp Pumped Dye Laser with a 480 Coumarin dye laser head designed and produced by Microoptical Engineering. Prior to marking, the fish were anaesthetized with MS 222, laid on their left sides and exposed to the air.

Each species was marked in a slightly different manner. Coho salmon received a single pulse of 230 millijoules irradiation with a l-mm diameter beam. Fall chinook salmon were marked by using a less intense beam (one pulse of 150 millijoules irradiation) that had a larger diameter (2 mm). To separate the effects of marking mortality from transport stress, a number of coho salmon (45) and fall chinook salmon (50) were not marked with the laser, but the adipose fins on these fish were clipped so they could be identified

The following eight different laser marks were tried: a single dot on the upper lobe of the caudal fin (single caudal); dots on the upper and lower lobes of the caudal (double caudal); a single dot on the upper shoulder (shoulder dot); two dots directly beneath the dorsal fin (double horizontal); two vertically positioned dots located anterior to the dorsal fin, with one placed above the lateral line and one below it (double vertical); a single dot on the anal fin (anal dot); and one or two dots on the operculum (opercule marks). The dot diameters ranged between 1 and 2 mm at the time of marking, except for the opercule marks which were larger and less well defined.

Opercule marks also healed rapidly, and along with the anal fin dots, became cryptic soon after lasing, consequently the underwater visibility of these two marks was not evaluated. After being marked, the fish were trucked back to the George Adams Hatchery, and each species was held separately in a 65-cm-diameter rearing tank.

Throughout a 90day holding period, less than 5% of the marked coho salmon perished; fall chinook salmon mortality on the other hand was around 55 %. Unlike the coho, these fish were smolting when they were lased and thus were easily stressed. Moreover, after being'marked, they were held in fresh water, and therefore had to revert back to a pair-like stage. We believe these two factors contributed significantly to their mortality. No growth-rate comparisons were made among fish having different laser marks, but mark retention was evaluated by examining each fish once every two weeks.

Fluorescent orange, red, and green elastomer marks were placed on fall chinook salmon at the George Adams Hatchery on the 16 July 1992 by NMT scientist Pete Bergman. Altogether 105 (70-l 10 mm) fish were marked (35 of each color). The fish were anaesthetized with MS-222 and a hypodermic syringe with a #25 needle was used to inject a 0.25-mm by 0.5-1.5-mm strand of colored elastomer under the adipose eyelid tissue. Mark retention and mortality records were not kept on these fish, nor were growth comparisons made.

Descriptions of the Assays Used--Two different procedures were created to appraise the underwater visibility of marked fish. In the first procedure, fish were observed at fixed distances from a viewing window (Fixed-distance Assay), while in the second procedure, fish were allowed to swim freely through out a portion of the chamber (Free-swimming Assay).

To start a Fixed-distance Assay, sets of two fish marked in the same way were collected from the holding tanks and placed into an aerated, 19 L bucket. Altogether 28 fish (16 laser marked, 4 adipose clipped, 6 elastomer marked, and 2 unmarked controls) were placed into the bucket. An observer would then blindly remove one of the fish and place it into a 24-cm-wide by 15.5-cm-high by 3.0-cm-wide Plexiglas box.

A V-shaped trough with an open bottom was placed over the top of the narrow holding box to facilitate this process. The holding box was half filled with water when a fish was placed into it. To help the box sink, and simultaneously provide fresh water to the secured fish, holes 6.25 mm in diameter were drilled into the upper half of the side walls but not the front and back walls. After fish insertion, the lid of the holding box was closed and the box was lowered into the viewing chamber by cords attached to each upper comer.

Initially, the holding box was placed 2.5 m away from one of the end viewing windows. Two observers independently examined a fish and attempted to identify its mark. If a mark was not recognized by both, the box was moved 0.25 m closer to the window, and another evaluation was made. When both observers felt that they had correctly identified a mark, the Plexiglas box was removed from the chamber, and the fish was closely examined to confirm its identification.

In this manner four assessments (two on each fish) were made of the underwater visibility of a mark type during a specific visibility trial. These data were used to generate a mean underwater visibility value. Six such trials were conducted, and in half of them the viewing box was left exposed to direct sunlight with light values in the chamber ranging from 900-1,600 fc. In the other half, plexiglass sheets were used to filter out overhead light and foot candle values ranged from 20 to 150.

Light values were determined by using a Kahlsico 23AM300 photometer and measuring light intensity in three standardized spots. The shaded and direct sunlight trials were paired, thus the same fish were used in each set even though they were conducted on adjacent days. This type of approach made it possible to determine if varying light levels influenced the visibility of marks

placed on the same fish. These paired evaluations occurred once every 2 weeks from early August through mid-September 1992. New fish were collected for each set of trials.

To conduct the Free-swimming Assay, the viewing chamber was divided into three segments (1.2 m X 1 m) by solidly colored Plexiglass panels. As in the Fixed-distance Assay, sets of fish having the same mark were collected and held in a single, 19-L bucket. Four fish were blindly removed from the bucket and placed into one of the viewing chamber sections.

An observer was given 5 minutes to identify the marks present on the fish. In addition, each observer ranked the relative visibility of each mark seen. At the conclusion of this test, fish were recaptured and carefully examined to confirm that proper identifications had been made and to discover the mark type present on any fish that were not identified. Only one of these trials was performed, and it was done while the chamber was exposed to direct sunlight (about 1,400 fc).

Results and Discussion

Retention of V.I. Tags, Adipose Clips, and Laser Marks--We did not quantitatively evaluate the retention of elastomer marks, but our impression was that none of the marked fish lost their injected elastomer strands. Moreover, these marks appeared to retain their color and size throughout our 89-day evaluation period. Laser marks on the other hand became enlarged, grew faint, and often disappeared entirely.

As mentioned above, all laser-marked fish were inspected at approximately biweekly intervals to determine the number of fish with each type of mark. A series of chi-square tests were conducted on these data to determine if some marks were disappearing more rapidly than others. Forty-six days after marking, we anaesthetized 162 lased coho salmon and carefully examined them for marks. All marks were in expected frequencies ($X^2 = 3.38 < X^2_{0.05,7} = 14.067$) except for those placed on the anal fin which had completely disappeared.

A similar appraisal on 164 lased coho salmon was conducted 67 days after marking. In this instance the Chi Square tests (overall $X^2 = 210.13 > X^2_{0.05,7} = 14.067$) showed that fewer opercule marks and a greater number of unmarked fish were present than expected. During the last examination, which took place 89 days after marking, 174 coho salmon were inspected. By that time (overall $X^2 = 755.49 > X^2_{0.05,7} = 14.067$) fewer laser marks, but more adipose clipped and unmarked individuals, occurred in the population than expected.

In aggregate, these analyses indicated that laser marks were disappearing from the marked population more rapidly than adipose clips and that some (e.g., anal fin and opercule dots) disappeared relatively rapidly. In general, laser-marked coho salmon retained their marks longer than those placed on fall chinook salmon. For instance, 46 days after marking had occurred, appreciably more unmarked fish and adipose clipped fish existed in the chinook population than expected ($X^2 = 31.82 > X^2_{0.05,7} = 14.067$; n = 138 fish) and no anal marks were. detected.

On sampling dates 67 ($X^2 = 260.77 > X^2_{0.05,7} = 14.067$; n = 124 fish) and 89 ($X^2 = 387.64 > X^2_{0.05,7} = 14.067$; n = 117 fish) increasing numbers of laser marks disappeared. Because of the relatively small numbers of marked chinook salmon remaining on those dates it was not possible to subdivide the chi-square tests enough to discern if marks, other than adipose fin clips and unmarked fish, were occurring in unexpected numbers. The data suggest, however, that both opercule and caudal fin marks were not as well retained as the other laser marks.

As previously discussed, further experimentation with the laser marking technique will have to occur before appropriate marking criteria (power, duration, and beam diameters) can be developed for small salmonids. **Marks** placed on body surface areas (double vertical, double horizontal, and shoulder dot) were initially very clear (jet black dots) and easily seen underwater. With time these began to fade, and in a number of cases double vertical marks joined together to create one large mark Marks placed on fins were often difficult to see and varied in color depending upon light conditions. They appeared as white dots when light was transmitted through the fin, but when viewed with reflected light these marks possessed a black center surrounded by a clear zone with a thin black border at its outside edge.

Results of the Fixed Distance Assay--Because laser marks were disappearing over time, our appraisals of their underwater visibility were biased, since we only examined fish that had recognizable marks. Consequently, our visibility estimates for these marks represent maximal values. Placing PIT tags (see Prentice et al. 1990a, b) into the fish at the time of lasing would allow assessment over a longer period of time (Desmond Maynard, NMFS, pers. comm.), and we plan to use this double marking procedure in future evaluations.

As described above, average visibility measurements were made for each type of mark during every observation period. These data were combined to create two overall values: one for full-sun and one for shaded conditions. In Table 2, these have been ranked by light type.

Of all the marks examined, adipose clips and double vertical laser marks were the most noticeable, whereas laser marks placed on the caudal fin were the least visible. Besides generating overall values, we also used these data to examine how mark visibility may have changed with time and whether some marks were more visible under full sunlight or shaded conditions.

We did not see any temporal decline in the visibility of adipose clips, elastomer marks, or laser marks placed on the caudal fin. On the other hand, double-horizontal and shoulder-dot marks became less visible with time mainly because 1) natural spotting along the back and upper-shoulder areas became more pronounced, and 2) the marks themselves tended to grow in size and lose color. Both of these factors reduced the amount of contrast that existed between normal integument coloration and the laser dots and thus diminished their visibility.

The type of light in the viewing chamber also apparently affected visibility. Elastomer marks, for example, appearedto be more visible under shaded conditions. This probably occurred because light was often reflected off the head when these fish were viewed in full sunlight, and this often made the elastomer colors difficult to see. Conversely, almost all of the laser marks (caudal marks, double vertical in chinook, and shoulder dots) were easier to see under bright light conditions.

Recall that these marks are made by damaged melanophores, which are unable to concentrate their melanosomes. This means that mark hue remains constant regardless of exterior light conditions. Under brightly lit circumstances unaffected parts of the body become pale to blend into the surrounding environment, but lased areas still remain black and thus marks become accentuated in full sunlight

The above observations were garnered from field notes and by examining temporal trends in the mean visibility values obtained on each mark. No statistical appraisals were conducted. However, the data that we gathered last summer and fall will be used to craft visibility experiments with known amounts of statistical power.

Table 2. Mean underwater visibility values of various marks placed on juvenile fall chinook salmon and coho salmon under full sunlight and shaded conditions in a viewing chamber.

Type of Mark	Species	Mean Underwater Visibility (in meters)
FULL SUNLIGHT		
Adipose Clip	Coho	2.50+[a]
Adipose Clip	Chinook	2.50+
Double Vertical Dots (Laser)	Coho	2.50+
Double Vertical Dots (Laser)	Chinook	2.50+
Shoulder Dot (Laser)	Coho	2.00
Double Horizontal Dots (Laser)	Coho	1.90
Grange Elastomer (V.I. Tag)	Chinook	1.89
Red Elastomer (V.I. Tag)	Chinook	1.84
Shoulder Dot (Laser)	Chinook	1.79
Double Horizontal Dots (Laser)	Chinook	1.79
Green Elastomer (V.I. Tag)	Chinook	1.56
Double Caudal Fin Dots (Laser)	Coho	1.46
Single Caudal Fin Dot (Laser)	Coho	0.87
SHADED LIGHT		
Adipose Clip	Chinook	2.46
Adipose Clip	Coho	2.43
Double Vertical Dots (Laser)	Coho	2.33
Double Horizontal Dots (Laser)	Chinook	2.13
Red Elastomer (V.I. Tag)	Chinook	1.92
Grange Elastomer (V.I. Tag)	Chinook	1.89
Shoulder Dot (Laser)	Chinook	1.71
Green Elastomer (V.I. Tag)	Chinook	1.65
Double Horizontal Coho (Laser)	Coho	1.63
Shoulder Dot (Laser)	Coho	1.54
Double Vertical Dots (Laser)	Chinook	1.50
Double Caudal Fin Dots (Laser)	Coho	1.29
Single Caudal Fin Dot (Laser)	Coho	0.63

[a] Mark was clearly seen at the maximal distance available in the viewing chamber.

Results of the Free-Swimming Assay--This assay had two objectives: 1) to determine the visibility of marks on free swimming fish, and 2) to identify which marks were the easiest to detect (i.e., to create an ordinal ranking of mark visibility). Only one assay of this type was performed, and that was done 90 days after the fish had been laser marked. We found that adipose clips, all the elastomer colors, and some laser marks (i.e., double vertical marks on coho and single shoulder dots on chinook) were highly visible on free-swimming fish. All the other marks (except two) could be detected but not as easily. The two that were not seen were double-horizontal and single-shoulder dots on juvenile coho salmon. In the confirmation part of this assay, all fish were held in the small Plexiglas viewing box used to conduct the Fixed-distance Assay and carefully examined. Even in this box, the two unidentified laser marks were not visible, suggesting that they had disappeared from the time we had selected them (3 days in advance of the assay) to when the test was performed.

This assay also indicated that identical marks need to be placed on both sides of a fish. In this study, marks were only present on one side; as a consequence, some marks could only be viewed for short periods of time because of fish orientation.

Quantitative assessments of underwater visibility and the behavioral effects of various visible marks on fishes have never, to our knowledge, been assessed. The field studies described above were done so that we could begin to formulate how such assessments could be rigorously conducted in the future.

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Section 11

PREDATION ON HATCHERY-REARED PACIFIC SALMON: POSSIBLE CAUSES OF VULNERABILITY; A LITERATURE REVIEW, SYNTHESIS, AND PRELIMINARY EXPERIMENTS

by

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Introduction

The purpose of this work was to provide baseline infotmation for a research proposal aimed at developing methodologies to mitigate the effects of predation by behavioral modification on selected species of hatchery-reared Pacific salmon (*Oncorhynchus* spp.). To this end, a literature review and synthesis was conducted to address some of the critical issues regarding predation of hatchery-produced juvenile Pacific salmon. In addition, preliminary experiments were performed to test the feasibility of conducting experiments to support the development of behavioral methodologies designed to improve the survival of Pacific salmon smolts to predation.

Two series of experiments were performed. The first series examined the effects of handling stress on predation vulnerability of coho salmon (0. *kisutch*) smolts. Results clearly demonstrated the efficacy of applying behavioral measures to assess the effects of stress induced by handling and transportation activities. Also of interest was the lack of linkage between plasma cortisol levels, a commonly applied measure of stress, and predation avoidance.

The second series of preliminary experiments examined the feasibility of conditioning spring chinook smolts to surrogate predatory stimuli as a way of increasing survival of fish subjected to predation. Other experiments have demonstrated that smolts that survived an initial exposure to predation have a significantly higher probability of surviving a second exposure to predation than did naive fish. However, our limited and preliminary attempts to condition spring chinook salmon (0. tshawytscha) smolts to surrogate predatory stimuli did not have a positive effect, i.e., there was no difference between naive and treated fish. Nonetheless, the fact that survival was improved following an exposure to predation provides evidence that surrogate predator conditioning may have the potential to be an effective method for reducing predator-induced mortality in this species.

Literature Review and Synthesis

Despite sharp increases in the number of smolts released from northwest hatcheries since the mid-1970s, there has not been an increase in numbers of returning adults. Although correlations have been made which strongly suggest that ocean conditions influence survival (Pearcy 1992), it has also been noted that return rates of hatchery fish are generally lower than those for wild runs (Raymond 1988). This suggests that hatchery-reared fish may be of a lower quality.

While great attention has been paid to improving smolt quality with respect to size and disease resistance, little attention has been directed towards the behavioral qualities of hatchery smolts. Since it is generally accepted that mortality from predators during smolt outmigration accounts for significant losses, it is pertinent to question whether hatchery smolts have decreased capabilities to avoid predation (Olla and Davis 1989, Olla et al. 1992). If this is the case, there are at least two possible causes.

On the one hand, selective breeding for traits which favor rapid growth and disease resistance may select inadvertently against behavioral traits which favor predator avoidance. If this is true, solutions will not come easily, for the science of dealing with the inheritance of behavioral traits is still very much in its infancy. On the other hand, the problem could be principally environmental, with the crowded, psychosensorydeprived world of the hatchery in some way either directly or indirectly inhibiting the full development of innate predator-avoidance skills (Olla and Davis 1989, Olla et al. 1992), or secondarily affecting social behaviors that mediate in predator

avoidance. If environmental conditions are responsible, solutions may be found by applying behavioral modification techniques to traditional rearing practices.

A second and closely related potential source of smolt mortality is stress. During handling and transport procedures associated with release into the environment or with diversion of fish around impediments to migration such as dams, smolts may experience levels of stress that impair their ability to behaviorally respond to challenges in the wild (Olla and Davis 1989, Olia et al. 1992). If predation is particularly intense during the first hours or days after release (Bayer 1986), stress associated with pre-release handling or transport may affect the ability of smolts to avoid predation (Olla and Davis 1989, Olla et al. in press).

In order to evaluate the potential of behavioral techniques designed to mitigate deficits in predator-avoidance skills or handling stress, it is important to clearly understand the fundamental nature of predator-prey interactions. With this in mind we made no attempt to examine the influence of disease and nutrition on the survival of hatchery smolts, as these topics are beyond the scope of this work. Instead, we concentrate on issues directly related to predation and interactions that may influence predator-prey dynamics.

For example, what are the major predators of salmon smolts and how have dams altered their effect upon smolt populations? We also examine differences between wild and hatchery stocks related to survival, growth, genetics, and behavioral attributes. In this way, we may be able to more clearly identify deficits in performance and develop approaches to investigate them.

In the following discussion, we examine these topics and summarize the pertinent literature. We then review the existing data on predator conditioning and evaluate its potential as a means for mitigating hatchery-induced deficits in predator-avoidance capabilities. Further, we discuss the utility of behavioral bioassays (Olla et al. 1980) with predation as an end point as a means for determining the effect of handling stress and how such procedures may aid in determining when and how to release smolts into the environment.

Following these analyses, we describe the results of preliminary experiments which we have conducted with coho salmon smolts addressing the influence of stress on predation vulnerability. We also present the results of preliminary experiments examining the feasibility of developing behavioral modification techniques that may be incorporated into existing hatchery management practices for predator conditioning chinook salmon smolts.

Clearly, predation is one of the major problems that must be understood and dealt with if hatcheries are to fulfill the mandate to increase dwindling salmon stocks. Numerous species prey upon sahnonid smolts during their residence in fresh water and during outmigration in fresh, estuarine, and marine waters. Birds and predacious fishes appear to take the greatest toll on juvenile salmonids (Jeppson and Platts 1959; Peterman and Gatto 1978; Wood 1985, 1986, 1987; Bayer 1986; Ruggerone 1986; Beamesderfer et al. 1990; Beamesderfer and Rieman 1991; Poe et al. 1991; Rieman et al. 1991; Vigg et al. 1991). In some cases, predation upon juveniles has been demonstrated as the limiting factor in adult returns. For example, reduction of predacious fish numbers in Cultus Lake, British Columbia, led to increases in the returns of adult sockeye salmon (0. *nerka*) (Foerster and Ricker 1941).

Under natural conditions, where predator-prey systems have coevolved, predators typically do not threaten the survival of salmon runs. For example, bird and fish predators typically display a functional response relationship to their prey such that predators are relatively inefficient at low prey abundances and proportional mortality of prey is low (Murdock and Oaten 1975, Peterman

and Gatto 1978). Hence, natural predator-prey relationships are typically stable (but see Peterman 1977).

However, on many river systems in the Pacific Northwest, particularly in the Columbia River Basin, dams have greatly altered predator-prey interactions. Not only do fish experience mortality as the result of passage through turbines and over spillways (Schoeneman et al. 1961, Ebel 1977, Rieman et al. 1991), but large still-water reservoirs increase the efficiency of resident predators.

For example, under natural stream and river conditions squawfish (*Ptychocheilus* spp.) do not prey heavily upon salmonids (Brown and Moyle 1981), since they typically occupy areas of lower water velocity (Faler et al. 1988) where they frequently do not encounter smolts migrating offshore (Hoar 1958). However, squawfish are opportunistic predators (Eggers et al. 1978, Poe et al. 1991) and their ability to prey upon young salmon is greatly increased in the slow moving waters of lakes and reservoirs (Beamesderfer et al. 1990, Poe et al. 1991, Vigg et al. 1991). This has resulted in large populations of squawfish and other piscine predators such as walleyes (Stizostedion vitreum) and smallmouth bass (Micropterus *dolomieui*) concentrating in reservoirs and below dams (Beamesderfer and Rieman 1991).

Rieman et al. (1991) estimated that from 1983-86, fish predators consumed 14% of all salmonid smolts entering the John Day Reservoir. Squawfish were estimated to account for 78% of this loss. Losses of this magnitude raise the question of whether these stocks have the behavioral capabilities to deal with such conditions. Unlike species which have coevolved with predators in lakes, many salmon stocks along the Columbia may lack well-developedbehaviors for mitigating predatory threat in slow-moving reservoirs. Studies of guppies (*Poecilla reticulata*) in Trinidad (Magurran and Seghers 1990, 1991) and European minnows (*Phoxinus shoxinus*) in England (Levesley and Magurran 1988, Magurran 1990) demonstrated that fish from populations sympatric with piscivorous predators have better developed innate antipredator behaviors than fish from populations where predators do not occur.

Reservoirs may also slow the passage of smolts (Bentley and Raymond 1976), resulting in predation for a longer period of time (Raymond 1979) and causing smolts to reach the lower reservoirs on the Columbia late in the season, when water temperatures are high (Raymond 1979, 1988). At high tempemtures, predatory fish are capable of consuming more smolts (smallmouth bass: Rogers and Burley 1991; northern squawfish: Vigg and Burley 1991). Rieman et al. (1991) estimated that losses to fish predators increased from 7% during June to 61% during August. Gulls (*Larus delawarensis*) also concentrate feeding upon dead and stunned fish that have passed through turbines and over spillways (Ruggerone 1986).

Data on the return of adult chinook salmon and steelhead to the Columbia River Basin generally show that wild fish have higher survival than hatchery fish (Raymond, 1988). Nickelson (1986) reached the same general conclusion for coho salmon in the Oregon Production Area. Such differences may be due in part to selective practices in hatcheries, and data indicate that wild and hatchery (domestic) stocks may also differ genetically.

Domestic salmonids often have lower genetic variability with respect to allozymes (Allendorf and Phelps 1980, Cross and Ring 1983, Vuorinen 1984, Vespoor 1988) and have diverged from their wild counterparts (Ryman and Stahl 1980). Similarly, there are morphological differences between domestic and hatchery fish that may have a genetic basis (Hjort and Schreck 1982, Taylor 1986, Fleming and Gross 1989), as well as differences in time of spawning (Ayerst 1977; Leider et al. 1984, 1990; Chilcote et al. 1986).

While there may be a question as to the adaptive significance of such differences, other differences have been noted that are arguably more closely related to survival. Hatchery selection has produced strains that grow rapidly under hatchery conditions, but which do not grow and survive as well in the wild as wild fish (Vincent 1960, Reisenbichler and McIntyre 1977). For example, when reared under identical conditions and then stocked into ponds, brook trout (*Salvelinus fontinalis*) derived from wild stock had significantly greater survival and growth than that of a domestic stock (Flick and Webster 1954).

In addition to growth-related differences, there are differences in behavior between wild and domestic stocks that are genetically based For example, domestic strains of brook trout have a tendency to position themselves near the surface of the water and are less likely to utilize benthic cover than are wild fish (Vincent 1960). Nor do domestic fish demonstrate the avoidance behaviors, often referred to as "timidness" or "shyness", that are characteristic of wild fish, even after months in the wild (Vincent 1960). Such deficits in behavior may make domestic fish more susceptible to predators.

Studies of the influence of ocean conditions on coho salmon survival suggest there may be differences in levels of predation upon hatchery and wild fish. Fisher and Pearcy (1988) argued that low returns of hatchery coho salmon from the Oregon Production Index area during years of poor upwelling (Nickelson 1986) are not caused by decreased growth or starvation of smolts, but are instead due to increased predation when other prey become scarce and predators switch to feeding on smolts (Bayer 1986). Interestingly, wild fish returns do not differ between years of good and poor upwelling (Nickelson 1986), and this may indicate that they are less vulnerable to changes in predation intensity. This may be attributable to differences in the size, timing, and duration of smolt runs or to differences in behavior between hatchery and wild fish.

Wild and domestic stock may also differ in other behaviors associated with social interactions that may ultimately influence predation vulnerability. Swain and Riddell (1990) found domestic coho salmon to be more aggressive than wild fish, and Moyle (1969) reported similar findings for brook trout. It has been well established that animals, including fish, balance feeding and other social behaviors against predatory risk (Caraco et al. 1980, Dill and Fraser 1984, Lendrem 1984, Magurran et al. 1985, Fraser and Huntingford 1986, Metcalfe et al. 1987, Huntingford et al. 1988, Ryer and Olla 1991). Thus, engaging in excessive agonistic behavior may be maladaptive, exposing hatchery fish to higher levels of predation.

While it is clear that genetic differences exist between hatchery and wild fish, it is also possible that deficits in behavior may be due to retarded development of innate capabilities, this being directly attributable to the hatchery environment (Olla et al. 1992). For instance, until release from a hatchery, salmonids are typically reared on an artificial diet in pellet form. Consequently, these fish lack the experience of feeding upon live prey. It has been suggested that this naivete may limit their ability to feed on natural forage after release, and thus contribute to mortality (Sosiak et al. 1979, Ersbak and Haase 1983, Suboski and Templeton 1989).

In contrast to these results from studies which have found hatchery-reared salmonids possessing the capability to rapidly switch to feeding upon live prey, e.g. coho salmon (Paszkowski and Olla 1985), rainbow trout (Bryan 1973) and Atlantic salmon (Suadmeyer and Thorpe 1987). However, there may be great individual variability in this capability. Paszkowski and Olla (1985) found that 3 1% of hatchery-reared coho salmon smolts did not feed at all after transfer from the hatchery to the laboratory, indicating difficulty in adapting to a changing environment. Similarly, 27% of tiger muskellunge failed to adapt to a diet of minnows after

14 days (Gillin et al. 1981) and it may take up to 30 days for Atlantic salmon smolts to start feeding after transfer from fresh to seawater (Usher et al. 1991). Other factors including selection of suboptimal diets (Sosiak et al. 1979, Meyers 1980, Ersbak and Haase 1983) and competition with wild fish (Dickson and MacCrimmon 1982, Bachman 1984) also may contribute to mortality in subtle ways that are not fully understood.

Just as hatchery fish have no experience in dealing with natural prey, they also have no experience in avoiding predators. There are, as far as we are aware, no valid studies that compare the predator avoidance capabilities of hatchery and wild fish. Such a comparison would be difficult to make. First, hatchery smolts are typically larger than wild smolts (Chapman 1962, Mason and Chapman 1965, Fenderson et al. 1968, Dill 1978, Berg and Northcote 1985), and most predators are size-selective (Barns 1967, Beall 1972, Hargreaves and LeBrasseur 1986). Second, wild smolts represent survivors of extensive predation during their freshwater residence. To compare wild fish, which have demonstrated their fitness by the fact that they have survived predation, with naive hatchery fish is not a valid test of the effect of rearing history upon predator-avoidance capability.

A second approach to examining hatchery-induced deficits in behavior is to determine whether remedial "predator conditioning" can improve predator avoidance capabilities in hatchery fish (Olla and Davis 1989). This approach involves training sessions during which a predator or simulated predator (the conditioned stimulus) is associated with an adverse unconditioned stimulus. Conditioned fish are then challenged with a living predator and their predator-avoidance capability is compared to that of unconditioned (naive) fish. Better survival of conditioned fish, as compared to unconditioned (naive) fish, may be considered as evidence of a behavioral deficit.

Unfortunately, few investigations testing the feasibility of this approach have been conducted. Thompson (1966) conducted predator conditioning with juvenile coho salmon and chinook salmon. Fry were conditioned to avoid a plastic model of a rainbow trout by means of electric shocks. Subsequent tests in aquaria with living rainbow trout revealed that conditioned fry experienced a 50% reduction in mortality.

Next, conditioned and unconditioned fish were released into a natural stream and collected from a weir down stream. Although survival differences between groups were small, a larger number of conditioned fish were recovered after running the gauntlet of natural predators. Further, when predators were elect&shed from the stream, two and one-half times more unconditioned than conditioned fish were recovered from stomachs.

Kanayama (1968), in a similar study, demonstrated that chum salmon fry could be conditioned with electric shock to avoid models of fish predators. When released into a seminatural stream with predators, more conditioned than unconditioned fish were recovered. Both of these studies (Thompson 1966, Kanayama 1968) share problems related to insufficient levels of replication, which compromise the ability to make generalizations. However, both studies suggest the promise of such techniques.

Employing a behavioral conditioning approach, Olla and Davis (1989) demonstrated in laboratory tests that predator avoidance performance of hatchery-reared coho salmon smolts can be improved by exposure to the visual, tactile, and olfactory cues associated with predation. Coho salmon smolts were introduced to a clear plexiglass enclosure positioned in the center of a large circular pool. Free-swimming lingcod (*Ophiodon elongatus*) were able to lunge at the fish from outside of the enclosure and a frozen lingcod, suspended from a pulley, was raised and dropped intermittently into the enclosure.

Chemical cues that might be associated with predation were provided from the effluent of a tank containing injured coho salmon smolts. Coho salmon smolts were exposed to such stimuli for 15 minutes, twice in a day, with a 2day interval between each exposure. Five days later the conditioned smolts were introduced to predator pools (without enclosures) along with an equal number of naive smolts. The result was that twice as many conditioned smolts as naive ones survived predation.

There is additional evidence that social facilitation of learning can play an important role in the acquisition of predator avoidance behaviors. Patten (1977) demonstrated that naive coho salmon fry, when in the presence of experienced fry (i.e., ones having experienced and survived predation), are better able to avoid predation. Similarly, Suboski et al. (1990) showed that zebra danio fish *(Bruchydanio rerio)* are able to learn, from association with trained fish, to associate predator avoidance behavior with a novel stimulus. These results suggest that if predator conditioning proves to be feasible approach to decreasing predation upon smolts, not all fish may need to undergo conditioning. It is possible that after release, conditioned fish may transfer their learned predator-avoidance behaviors, via social facilitation, to unconditioned fish.

Another factor relevant to predation loss deals with stress. Numerous studies have demonstrated that injury, physical exercise, handling, and transport may produce physiological changes in freshwater fishes (Houston et al. 1971a, b; Miles et al. 1974; Barton et al. 1980; Barton and Peter 1982; Pickering et al. 1982; Carmichael et al. 1984; Woodward and Strange 1987). The most widely adopted technique for tracking the course of such physiological changes is to monitor levels of corticosteroids (Houston et al. 1971a, Strange et al. 1977, Strange and Schreck 1978, Barton and Peter 1982).

Judged by this criterion, salmon appear to recover from such stresses in times ranging from 3 to 24 hours (Strange et al. 1977, Schreck 1981, Barton et al. 1986, Redding and Schreck 1983). Although elevated levels of corticosteroids have been associated with deficits in performance (Congleton and Wagner 1988, Franklin et al. 1992, for recent review see Schreck 1990), the ecological consequences of such physiological changes have been largely overlooked. Stress may render juvenile fish more vulnerable to predation (Sylvester 1972, Coutant et al. 1979).

Addressing this problem, Olla and Davis (1989) have developed a "behavioral bioassay" as a means of determining when fish have recovered from stress. They found that coho salmon smolts returned to their pre-stress predator avoidance capability within 90 minutes of being stressed. In a second study (Olla et al. in press), coho salmon recovered their behavioral ability to deal with predators within 90 minutes of being stressed, even through cortisol levels remained elevated for more than 4 hours. These results suggested that at least one stock of coho salmon smolts were capable of behaviorally responding to predator challenges long before they had returned to nominal physiological levels. However, it is clear that different stocks may possess very different capabilities of responding to stress, as our preliminary experiments indicated.

In the following pages, we describe results of the preliminary experiments we conducted with coho salmon and spring chinook salmon smolts as part of a feasibility study for the NATURES program. Our experiments with coho salmon examined the role of stress in rendering smolts more vulnerable to predation; our experiments with chinook salmon addressed the feasibility of conditioning smolts to avoid predation and the feasibility of utilizing surrogate predatory stimuli as part of a conditioning program.

Preliminary Experiments Effects of Stress on Vulnerability to Predation in Cobo Salmon Smolts

The aim of these experiments was to quantify the effects of handling stress on predator avoidance, plasma cortisol concentrations, and nonpredator-induced mortality of coho salmon smolts. The results of these experiments were compared with published results on a different stock of coho smolts (Olla et al. in press).

Materials and Methods

The coho salmon smolts used were hatchery-reared (Salmon River Hatchery of the Oregon Department of Fish and Wildlife, originating from Fall Creek stock), age-l fish that had smolted in the spring of 1992. Groups of 200-500 fish, ranging in length from 90 to 140 mm, were held in circular tanks (2.0-m diameter, 0.86-m depth, 2.70-m^3 volume), each supplied with flowing seawater (30-33‰, 10-13°C). Fish were fed moist Biodiet pellets every other day for l-3 months prior to testing. Lingcod, *Ophiodon elonqatus* (45-70 cm long) predators were obtained by hook-and-line fishing off of Newport, Oregon. Predation experiments were conducted from May through July in four plastic-lined circular pools (4.54-m diameter, 0.9 15-m depth, 14.8 1 -m^3 volume), each supplied with flowing seawater (30-33‰, 10-13°C). One lingcod resided in each pool and fed on 10-12 coho smolts weekly during predation trials. One trial per pool per week was conducted, with this interval determined by the time for recovery of lingcod appetite.

One day before a trial, 10 coho smolts that were to serve as unstressed control fish were placed into each of two opaque enclosures (0.60-m diameter, 0.80-m depth, 0.23-m^3 volume) within an experimental pool. These opaque enclosures were supplied with seawater recirculated from the experimental pools by air-lift pumps. In alternate trials, control or stressed groups of coho salmon smolts were either marked for later identification by clipping the adipose fins or handled as if marking.

On the day of a trial, fish to be stressed were transferred to a 20-L bucket with a mesh bottom and held out of the water for periods of two 1 minute bouts separated by a 90-minute recovery period and followed by a 1 minute bout or a 30 second bout. Ten fish were then distributed to each of the two opaque enclosures that already held control fish in the predator pool. Following a recovery period of either 4 hours (six trials) or 24 hours (six trials), fish in one opaque enclosure were released into the predator pool and the fish in the other enclosure were carefully netted and killed with buffered tricaine (MS-222) for plasma cortisol analysis.

Smolts were exposed to lingcod predation for 15 minutes or until half were eaten. Survivors were then netted and identified. Blood was sampled immediately from fish killed in anesthesia by severing the caudal peduncle and collecting blood into heparinized capillary tubes. The plasma was separated from other constituents by centrifugation and stored at -20°C for later analysis. Cortisol concentrations in the samples were determined with radioimmunoassay techniques as described by Foster and Dunn (1974) and modified by Redding et al. (1984). Values for cortisol concentration obtained from five unstressed fish and five stressed fish per replicate trial were pooled to give a mean value per treatment per replicate.

Values for cortisol concentration, number of fish eaten and fish mortality did not fit normal distributions, so nonparametric statistical methods were applied. To assure that appetite was not a factor in the analysis, the sign test (Conover 1980) was used to test differences in numbers eaten between unstressed and stressed fish based on results from trial pairs within a recovery time. The

Tukey two-sample test (Tukey 1959) was used to test differences in concentration of cortisol and mortality between stressed and unstressed fish within a recovery time.

Results

Four hours after administration of either two l-minute, one l-minute, or one 30-second stress, no significant stress-related mortality was detected ($P > 0.05$, Fig. 1 l-l). However, after 24 hours, the first two treatments had induced significant mortality ($P < 0.05$), while the third had not ($P > 0.05$, Fig. 11-l).

The effects of stress were clearly manifested in vulnerability to predation. For all stress treatments, significantly more stressed fish were eaten 4 hours after being stressed ($P < 0.05$, Fig. 1 l-2). Predation trials 24 hours following stress could not be performed on smolts stressed for two l-minute bouts or a single l-minute bout because of the high level of mortality which was induced by stressing procedures. However, fish stressed for 30 seconds showed recovery after 24 hours, with the percent of fish eaten not differing significantly from controls ($P > 0.05$, Fig. 1 l-2).

For all stress treatments, plasma cortisol concentration was significantly higher ($P < 0.05$) in fish after 4 hours. Although plasma cortisol levels for fish stressed for 30 seconds decreased significantly after 24 hours ($P < 0.05$, Fig. 1 1-2), these levels still remained higher than for controls (P c 0.05, Fig. 11- 2).

Discussion

It appeared that handling stress was associated with a short-term predator avoidance deficit that ended within 24 hours after a 30-second stress, while significant mortality resulted within 24 hours from two l-minute stresses or a single 1-min stress. In earlier studies with a different stock of coho salmon smolts from Oregon Aqua Foods, we found that recovery from a predator avoidance deficit occurred within 4 hours after a l-minute handling stress (Olla and Davis 1989, Olla et al. in press). Also in contrast to the present work, no stress-induced mortality was observed by Olla and Davis (1989) after 24 hours.

As in an earlier study with coho salmon smolts (Olla et al. in press), no clear relationship was ascertained between cortisol concentration and predator avoidance ability. The use of cortisol as a standard bioassay of the potential for survival of smolts needs further evaluation.

It is also apparent that there were marked differences between coho salmon stocks in their ability to recover predator avoidance ability after a handling stress. Whether these differences were associated with genetic or environmental factors was beyond the scope of this study and should be evaluated further.

Acute stresses such as handling and transport can have behavioral effects. Unstressed coho salmon juveniles learned to recognize selected odorants in a few days, but required 5-7 weeks to do so after imposition of transportation stress (Sandoval 1979). Additionally, coho salmon trained to recognize odorants required 2 days of recovery from a short handling stress before regaining their original level of performance. Few studies have evaluated ecologically meaningful effect of acute stress on behavior and correlated these effects with biochemical measures (Olla et al. in press).

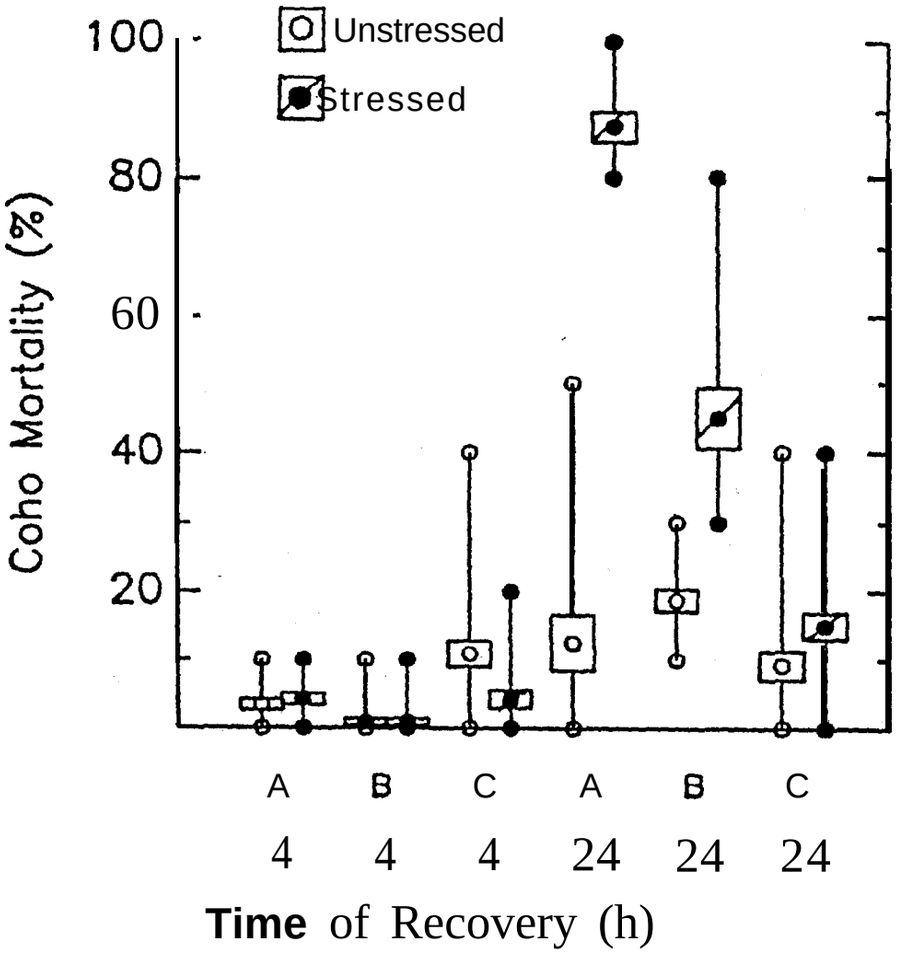

Figure 1 l-l. Effect of handling stress and recovery time on mortality (%) of unstressed and stressed coho salmon smolts. Handling stress was (A) two I-minute stresses, (B) a single l-minute stress; and (C) a 30 second stress. Recovery times were 4 and 24 hours. Box plots represent median, upper and lower quartile, and range.

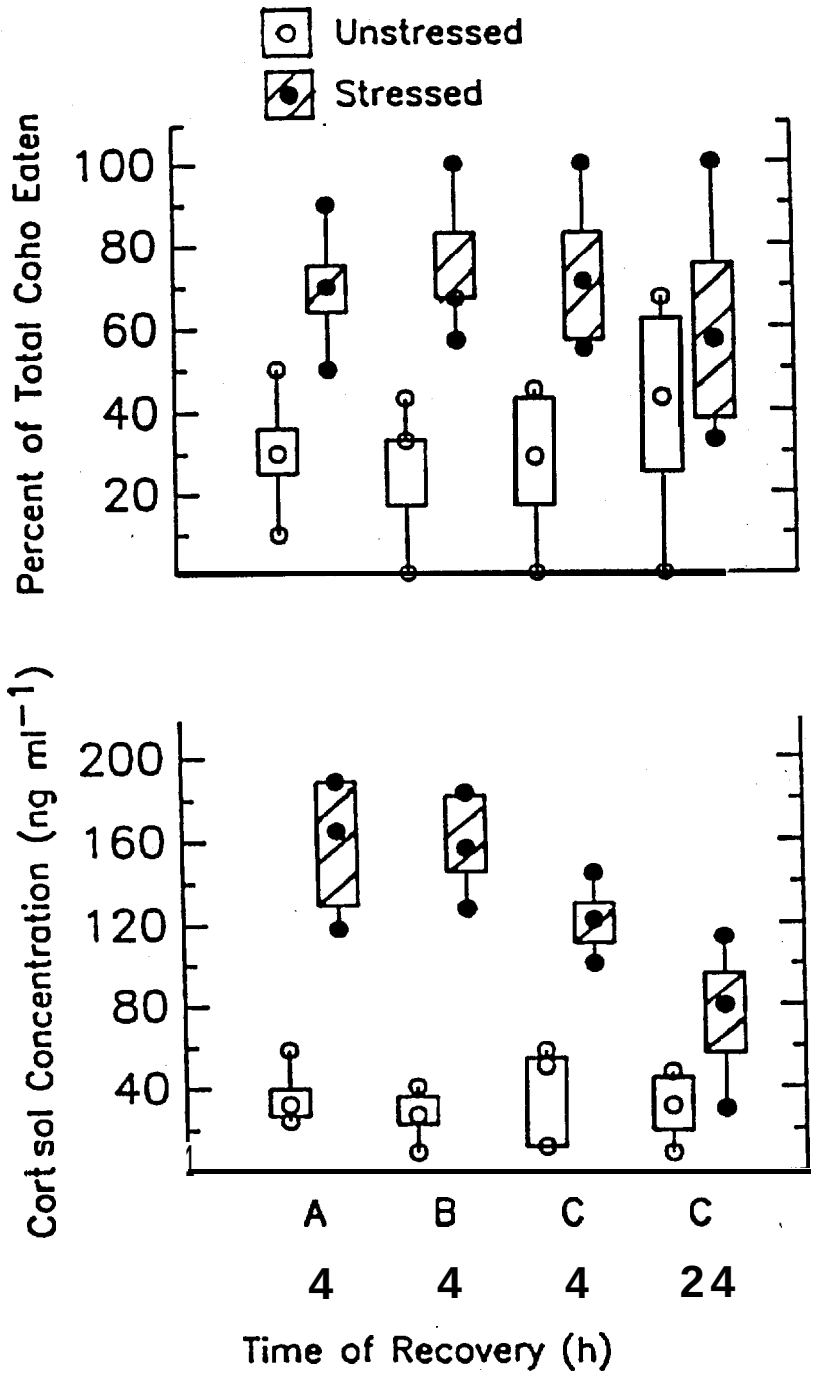

Figure 11-2. Effect of handling stress and recovery time on percent of total coho salmon smolts eaten and cortisol concentration (ng ml^{-1}) of unstressed and stressed smolts. Handling stress was (A) two l-minute stresses, (B) a single l-minute stress, and (C) a 30 second stress. Recovery times were 4 and 24 hours. Box plots represent median, upper and lower quartile, and range.

Although some behaviors can be affected by stress for days and even weeks after a stressful event, our results indicated that some basic, behaviorally-mediated survival skills associated with avoiding predation could be recovered within 4-24 hours (Olla and Davis 1989; Olla et al. in press; this study). However, the intensity and duration of the stress would play a role in the magnitude of behavioral performance deficits because the physiological effects of multiple stresses have been shown to be cumulative (Barton et al. 1986, Maule et al. 1988, Mesa 1989). Effects of multiple stressors were not evaluated in this study because of the mortality we observed, but we conjecture that recovery of predator-avoidance skills would take longer after exposure to more intense or multiple stressors such as those imposed during transport of smolts from hatcheries for outmigration.

Feasibility of Conditioning Spring Chinook Smolts to Surrogate Predatory Stimuli

The aim of these experiments was to evaluate the potential of using surrogate predatory stimuli in conditioning spring chinook smolts to avoid predation. An initial experiment was conducted to confirm that spring chinook salmon smolts could be conditioned to avoid predation, using lingcod as a predator and following the procedures of an earlier study on coho salmon smolts (Olla and Davis 1989). Subsequent experiments were conducted to evaluate the effectiveness of various surrogate predatory stimuli in conditioning spring chinook salmon smolts.

Materials and Methods

The spring chinook salmon smolts used in these experiments were hatchery-reared (Oregon Department of Fish and Wildlife Willamette Hatchery, originating from Willamette River stocks), age-l fish that had smolted in fall 1991. Groups of 200-500 fish, ranging in length from 120-210 mm, were held in circular tanks (2.0-m diameter, 0.86-m depth, 2.70-m^3 volume) supplied with flowing seawater (30-33‰, 10-13°C). Fish were fed moist Biodiet pellets every other day for l-3 months prior to testing.

Lingcod predators were 45-70 cm long and were obtained by hook-and-line fishing off of Newport, Oregon. All predation experiments were conducted from December through March in three plastic-lined circular pools (4.54-m diameter, 0.915-m depth, 14.81-m^3 volume), each supplied with flowing seawater (30-33‰, 10-13°C). One lingcod resided in each pool and fed on 10- 12 chinook smolts weekly during predation trials. One trial per pool per week was conducted, with this interval determined by the time for recovery of lingcod appetite. A fourth pool was used as a conditioning tank to test whether exposure to surrogate predatory stimuli could increase survival to subsequent predation. Chinook salmon smolts were startled in the tank with a net apparatus (see below).

Following an experimental protocol that had been previously used on coho salmon smolts (Olla and Davis 1989), we mixed chinook salmon juvenile survivors of lingcod predation with naive fish to determine the effect of prior experience with a predator on survival. Predation trials were conducted as previously described.

We preformed two groups of "training" sessions in which chinook salmon were mechanically conditioned to surrogate predatory stimuli with increasing levels of intensity. A training session consisted of dropping a net (2-m diameter) with attached objects (10 x 20 cm) on a pulley system into the tank, followed within 10 second by dropping of a weighted model lingcod

70-cm length and also on a pulley system into the tank. These objects were vigorously moved in the tank for 45 seconds and then retrieved This sequence was repeated every minute for 10 minutes.

In the first group of training sessions (n = 6 trials), we conducted three sessions, each separated by 2 hours and followed by 2 days of recovery. In the second group of training sessions (n = 4 trials) we conducted 6 sessions, each separated by 2 hours, followed by 2 days of recovery, and then followed by an additional 6 sessions for 1 day, followed by an additional 2 days of recovery. Effluent from wounded chinook salmon was introduced to the tanks prior to each training session.

During recovery periods, 10 treated fish from each trial were placed in an opaque enclosure (0.60-m diameter, 0.80-m depth, 0.23-d volume) within one of the experimental pools. The opaque enclosures were supplied with seawater recirculated from the experimental pools by air-lift pumps. Following the final 2 days of recovery, 10 naive fish were added to the treated fish in this enclosure. The treated groups of fish were marked for later identification by clipping the adipose fins whereas control fish were handled without the marking.

On the day of the predation trial, fish in the enclosure were released, and predation was allowed to continue for 15 minutes or until half of the fish had been eaten, with survivors then netted and identified.

Results

It appeared that experience played a role in predator avoidance. Naive spring chinook smolts that had not been previously exposed to predation were eaten at a significantly higher rate than those that had survived a previous exposure to predation ($P < 0.05$, Fig. 1 l-3).

Employing a conditioning regimen using surrogate predatory stimuli resulted in no net improvement in predator avoidance of experienced fish over naive fish ($P > 0.05$, Fig. 1 l-4).

Discussion

Spring chinook salmon smolts that survived one exposure to lingcod predation had a higher probability of surviving a second exposure to predation than did naive fish. This was in agreement with an earlier study using coho salmon smolts (Olla and Davis 1989). The positive response obtained indicated that spring chinook smolts may possess the capability of being conditioned to predators;

While our preliminary attempts to condition these smolts to surrogate predatory stimuli did not have a positive effect, i.e., no difference was observed between naive and treated fish, the potential for predator-avoidance training this species obviously exists. We suspect that smolts in our study were allowed partial refuge from aversive stimuli during conditioning, and therefore they were not able to link the presence of such stimuli with the threat of predators. Future research with conditioning of spring chinook salmon smolts to surrogate predatory stimuli should include evaluation of electrified predator models and conditioning apparatus that ensures that all fish are exposed to stimuli throughout the entire conditioning tank (i.e., no refuges present).

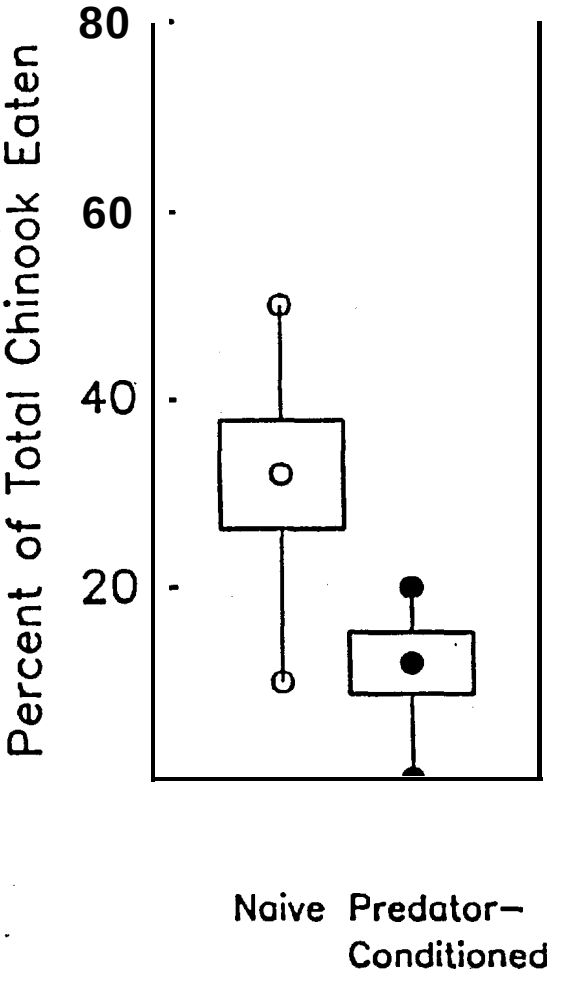

Figure 1 l-3. Effect of prior experience with lingcod predation on percent of total spring chinook salmon molts eaten. Smolts were naive or predator-conditioned. Box plots represent median, upper and lower quartile, and range.

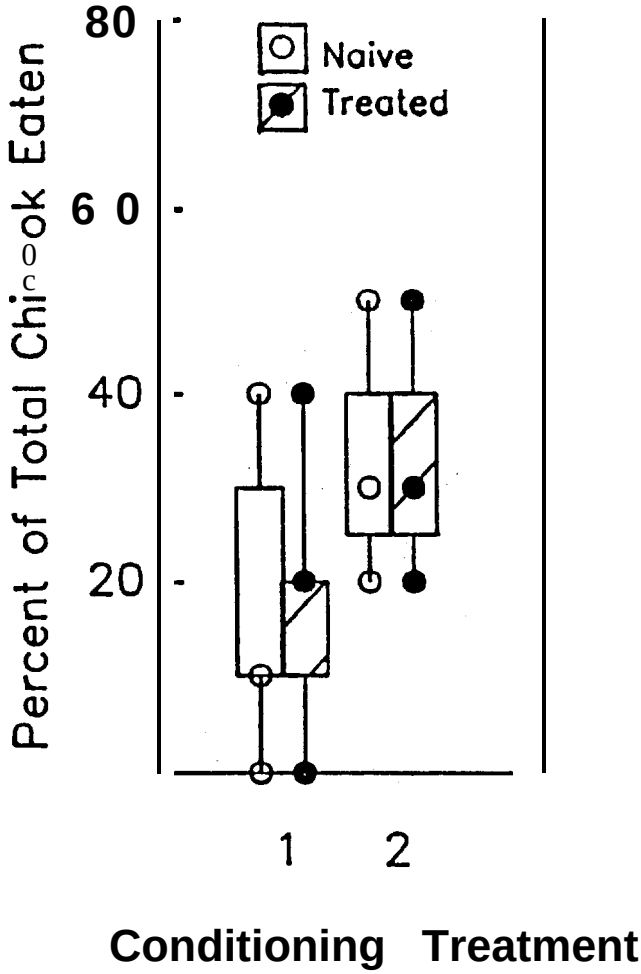

Figure 1 l-4. Effect of conditioning to surrogate predatory stimuli on percent of total spring chinook salmon smolts eaten. Conditioning treatments included (1) 1 day of conditioning and (2) 2 days of conditioriing (see methods). Box plots represent median, upper and lower quartile, and range.

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Section 12

REVIEW OF FEEDS AND FEED DELIVERY SYSTEMS SUITABLE FOR THE NATURES PROGRAM

by

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Contents

Introduction

The National Marine Fisheries Service (NMFS) is developing and testing a natural rearing enhancement system (NATURES) concept to produce hatchery-reared Pacific salmon (Oncorhynchus *spp.*) that are morphological, physiological, and behavioral equivalent to their wild-reared counterparts (see Sections l-l 1 of this report). The U.S. Fish and Wildlife Service, Abernathy Salmon Culture Technology Center, was subcontracted by NMFS to investigate feed and feeding technologies for use in the NATURES project. In the first phase of this project, we reviewed selected fisheries literature on 1) wild salmonid foraging behavior, 2) influence of hatchery rearing on the development of foraging skills, and 3) the historical precedent for subsurface feeding of fish.

Major findings from this search are summarized in the attached literature review. The results from this literature review, and from surveying manufactures of formulated feeds, were used to generate an outline for development of a feed/feeding system for the NATURES project that will emulate the drifting patterns of invertebrate prey in a stream system.

Development of formulated diets for many cultured species has revolutionized the aquaculture industry by allowing for intensive production due to a reliable supply of nutritionally balanced feed. Many hatchery programs have embraced this technology and standard hatchery practices have developed feeding protocols around pelleted diets. Feed program development efforts have focused on improving feed quality, optimizing feed conversion, automation of the feed delivery process, and assessing the effects of diet on fish health.

Yet, comparatively little effort has been devoted to refining techniques for presenting feed more naturally, despite compelling evidence that prey movement, size, and shape trigger the initial feeding response in juvenile salmonids (Irvine & Northcote 1983). Further, it has been demonstrated that taste (Stradmeyer 1989, Atema 1980), smell (Atema 1980), and texture (Stradmeyer et al. 1988) are key elements in determining rejection or acceptance of a diet.

Current hatchery practices tend to produce fish that are surface oriented, conditioned to feeding on a non-evasive homogenous-mass of pellets, and accustomed to approaching large moving objects on the surface. This conditioning produces an aggressive fish with inflexible foraging skills and a predisposition to avian predation. In situations where stocks are raised in a hatchery for ultimate release into the wild (supplementation programs), modifications to the way feed is presented could be a critical factor in developing foraging behavior patterns that emulate those of wild salmonids. Differences in foraging flexibility (Sosiak et al. 1979, Ersbak and Haase 1983) and use of cover (Bachman 1984) have been identified as key factors effecting survival of hatchery stocks after release.

In order to develop alternative feeds and feeding systems for hatchery-reared stocks, it is important to review natural foraging behavior in wild salmonids to determine which aspects of the predator-prey relationship or natural environment lend themselves to artificial manipulation. The purpose of this literature review is to identify primary foraging patterns in target salmonids, compare and contrast this behavior with that of hatchery stocks, and identify available technologies that can be adapted to present feed naturally.

Foraging Behavior of Juvenile Salmonids

The behavioral responses of wild juvenile salmonids to prey indicate that they are visual feeders (Blaxter 1980) that dart out from cover to seize invertebrates drifting in the stream current and then return to their feeding station (Stradmeyer and Thorpe 1987, Keenleyside 1962). After capture, prey is manipulated in the buccal cavity to evaluate palatability and to position it for swallowing. At this stage, prey is either ingested or rejected (Wankowski 1979).

Vision

Studies by Brett and Ali (1958) indicate that the salmonid eye is adapted for feeding and navigation under day and night conditions. The configuration of cones and rods are typical of vertebrates with average visual acuity. As in many teleosts, the salmonid eye adapts for functioning under light and dark conditions. Light adaptation is characterized by cone vision, high visual acuity, low sensitivity to light conditions, and color vision, while adaptation to dark is characterized by the opposite (e.g., low visual acuity and high sensitivity) (Blaxter 1980).

Brett and Groot (1963) found that juvenile coho salmon (0. *kisutch*) could feed on *Daphnia* with a 95% success in light ranges from 1 x 10^{9} to 1 x 10^{-1} fc with feeding success decreasing rapidly to the extinction point at 1 x 10^{-5} fc. This data revealed that salmonids can visually feed at light levels found at night, but feeding efficiency diminished quickly after dark due to reduced acuity. Subjects could not feed in complete darkness, indicating that olfaction and tactile senses were not developed sufficiently to allow feeding without the use of vision. The authors speculated that vision was the primary sense used in feeding for salmonids.

Studies reviewed by Blaxter (1980) indicate that visual acuity improves with age. Due to reduced sensitivity of the eye in young fry, they must rely on prey characteristics such as visual contrast with the environment, scent, proximity, and movement as the key elements in successfully locating and capturing prey until vision improves to the point where individual prey images can be distinguished.

Olfaction and Taste

While vision is recognized as the primary sense used in feeding, there is evidence to suggest that olfaction and taste also play a key role in prey selection and possibly detection. McBride et al. (1962) found that sockeye salmon (0. *nerka*) exhibited search behavior when aqueous extracts of common food items (concentration 12.5 µg/L) were injected into the water current. The subjects were selective in their responses, only reacting to extracts from feeds they had been conditioned to feed on for 1 week

Atema (1980) speculated that fish develop "olfactory imprints" of their environment which enable them to identify familiar prey before encountering it visually. Brett and McKinnon (1954), as cited by Brett and Groot (1963), found that coho salmon and chinook salmon (0. *tshawytscha*) demonstrated alarm reactions when washes from predatory mammalian skins were placed in the water, highlighting the importance of olfaction in evaluating the surrounding environment.

Studies on olfactory imprinting of juvenile salmonids to their native streams indicates individuals can detect minute variations in water chemistry, enabling them to distinguish a chemical gradient in the water body that leads them to the correct spawning stream (Wisby and Hasler 1954, Hasler 1954, Hasler and Scholz 1980). Hasler and Scholz (1980) reviewed studies on the artificial imprinting of salmonids to a stream using morpholine. Their data suggested that it is possible to

minimize straying of hatchery stocks or redirect the path of the spawning migration through the use of these compounds. It would stand to reason that similar olfactory training could be provided to hatchery stocks to improve tracking of prey by establishing "olfactory imprints" of common natural feeds.

Prey Movement

Movement enhances the ability to visually detect prey that is otherwise hidden through cryptic coloration or behavior patterns by improving contrast with the surrounding environment. Rimmer and Powers (1978) observed that Atlantic salmon (*Salmo salar*) alevins would only consume prey that was nearby and in motion, and that stationary items placed within the visual field were ignored. Iwai (1980) suggested that prey motion also stimulates body-surface neuromasts (precursors to the lateral line system), alerting the alevins to prey within capture range. They concluded that prey motion was important but could be provided by either the water current or individual motion of prey items.

Rimmer and Powers (1978) cited work by Stuart (1953) where Atlantic salmon were fed live and dead mayfly nymphs: the fry consistently fed on live prey when the only noticeable motion of these subjects was the occasional vibration of the gill lamellae. This data suggests that the ability to cue into prey-specific movement patterns could play a role in foraging success.

Habitat Preferences

Lister and Genoe (1970) summarized post emergent feeding behavior of chinook salmon in terms of habitat selection as follows: Initially, fry hid in the interstitial spaces of the gravel substrate feeding on planktonic drift and periphyton. This stage was followed by migration to areas of low stream velocity, where feeding stations were established in parts of the stream with cover along the bank. As the fry grew, they migrated back to the deep, high-velocity portions of the stream feeding on invertebrate drift dislodged by the current,

Mundie et al. (1990) observed similar behavior in coho salmon fry released into the downstream end of a pool in a seminatural rearing channel: the fish immediately swam into the gravel substrate in the riffle zone, emerging a day or two later at the stream margin under cover.

Sager and Glova (1987) studied the prey preferences of juvenile chinook salmon transplanted to New Zealand streams and found the stock developed feeding behavior similar to their North American counterparts. Their observations indicated that specimens smaller than 55 mm preferred marginal, slow-moving water with adequate cover, while those greater than 55 mm preferred deep-water pools.

Keenleyside (1962) observed that wild juvenile Atlantic salmon fed equally well from the bottom and from drifting prey in the water column. As parr grew and moved into deeper, faster water, the focus of feeding shifted to drifting invertebrates. Keenleyside (1962) also found that juvenile Atlantic salmon adjusted their distance above the substrate relative to the stream velocity: the stronger the current, the more body contact they had with the substrate--to the point of partially burying their bodies in areas with strong currents. Fish tended to use instream rocks for cover and hold position except when striking prey. The overall strategy appears to be one of energy conservation in a physically demanding environment.

Diel and Seasonal Variation in Feeding Patterns

Eriksson and Alanärä (1990) reviewed studies related to the daily and seasonal rhythms of wild and captive salmonids. Their data suggest that stream-dwelling salmonids pattern their feeding rhythms to seasonal and diurnal variations in invertebrate drifting patterns. For instance, stream-dwelling salmonids tend to feed at dawn and dusk in spring and early summer, corresponding with the peak availability of drifting benthic invertebrates. In late summer and fall, invertebrate production decreases, but the abundance of surfacedrifting terrestrial insects increases. Juvenile salmonids shift to a pattern of surface feeding by day to better utilize this resource.

Eriksson's (1973, 1975, 1978) earlier studies indicated that many salmonids are crepuscular feeders (dawn and dusk feeding), but that there is considerable variation with season on the intensity of feeding at either dawn or dusk.

Eriksson and Alanärä (1990) cited a study by Landless (1976), which demonstrated seasonal and diurnal peaks in the feeding activity of captive rainbow trout (0. *mykiss*) allowed to voluntarily select feeding times through the use of an electronic demand feeder. The feeding pattern selected strongly resembled the natural feeding rhythms seen in wild fish and suggests that seasonal variations in feeding cycles could be under genetic control. If this is the case, then it would be advantageous to develop feeding systems capable of mimicking the spatial and temporal variations in food availability found in natural ecosystems.

Prey Preference

Sager and Glova (1987), studying juvenile chinook salmon, observed that diet varied seasonally with a tendency for the dominant invertebrates in the drift to make up a corresponding large part of the diet. This finding supports the theory that salmonids are primarily opportunistic feeders. However, there was some evidence of selective predation (i.e., the prevalence of chironomid larvae, coleopterans and uichopteran imagos in stomach contents exceeded that in the drift samples). The authors speculated these changes could be due to differential drifting behavior of these prey organisms, which made them more attractive or available to the fish.

Galbraith (1967) found that rainbow trout feeding on *Daphnia* were highly selective for size, taking very few specimens less than 1.3 mm despite an abundance 'of smaller organisms in plankton tows. Gillraker measurements revealed .a mean gap of 1.1 mm, which indicate that some filtration effect was at work sorting zooplankton. There were enough smaller Daphnia in the stomach contents to indicate that selection processes other than filtration were being utilized to obtain optimal forage.

Craddock et al. (1976) found that migrant juvenile chinook salmon in the lower Columbia River fed heavily on Daphnia from July to September. Up to 98% of stomach contents consisted of this genus and analysis revealed a preference for larger specimens, ranging in size from 1.3 mm to 2.2 mm, and thus confirming Galbraith's findings. English (1983) found that 6.5-g juvenile chinook salmon placed in situ in containers actively fed on marine zooplankton, with growth rates of fish ranging from 3.9% body weight per day (BWD) to -0.5% BWD, depending on prey availability. They were highly selective for high-contrast prey (i.e., dark eye or intestinal track) in the size range of 1.4 to 4.5 mm.

English (1983) estimated that juvenile salmonids can successfully search 2.3 m^3/hr when feeding on zooplankton. Wankowski (1979) evaluated a number of morphological features of

juvenile **Atlantic salmon** and determined that spacing between gillrakers determined the minimum size of particle that could be filtered, while mouth width determined the maximum size of prey that could be consumed. Subjects evaluated in these trials varied in size from 3.5 to 20.0 cm. In this fish size range, the calculated constant **for** maximum prey size was 0.06 times fish fork length. In tests of the model, Wankowski found that subjects fed prey above and below the threshold sizes lost weight and had no stomach contents, indicating that the model was accurate.

Effects of Prey Density on Foraging

Slaney and Northcote (1974) found that juvenile rainbow **trout** allowed to voluntarily establish feeding territories in a test stream tended to require less space as the density of prey items increased There was a corresponding reduction in aggression with increased feed availability. Territory requirements also decreased when the individuals were closer to the source of feed. These observations presuppose strong competition for optimal foraging locations and a willingness to compromise space requirements for improved feed availability. These behaviors suggest that a feeding system should **provide** a number of **"optimal"** feeding territories within the rearing structure to improve stock distribution.

One approach that we considered for training captive stocks to emulate the feeding behavior of wild **fish** would be to deliver the hatchery diet or natural feed items in a mechanically generated, subsurface, water current that simulates invertebrate drift patterns. A critical element in evaluating the. **effectiveness** of prototype "subsurface" feeders will be to determine at what density fish stocks shift from a natural pattern of territorial feeding using cover to one of "free for all" schooling behavior typical of intensive hatchery culture operations. It might be beneficial to create a multi-leveled environment within the rearing structure to promote use of territory and reduce aggression while maintaining high densities.

Feeding Behavior of Hatchery vs. Wild Salmonids

Supplemental Feeding

Irvine and Baily (1992) evaluated the effects of **instream** supplemental feeding on the growth of wild and hatchery-planted **coho** salmon stocks. Hatchery stocks in supplemented sections had better growth rates than sympatrically raised wild fish, but fish of both stocks in supplemented sections had better growth rates than their cohorts in unsupplemented sections. In unsupplemented sections, wild and hatchery fish grew at similar rates, but the wild fish had a better condition factor. Their comparisons of growth and condition factors for a stream section containing all wild fish and a segment with mixed hatchery/wild fish indicated that introductions of hatchery fish did not adversely effect the foraging success of wild stocks. The authors observed a drop in population for the supplemented site after several months, corresponding with an increase in the number of avian predators. They noted that fry emerged from cover for the formulated diet, consequently becoming less evasive as the experiment progressed These observations imply that breakdowns in cryptic behavior due to a surface oriented feeding pattern are brought on by conditioning, as both wild and hatchery stock responded in a similar way.

Mundie et al. (1990) compared survival to adulthood in groups of **coho** salmon raised in a seminatural stream channel or by standard hatchery practices. Results indicated that hatchery methods produced better survival. Fish raised in the seminatural stream were afforded the benefit of partially feeding on natural prey and exposure to complex habitats before release. Automatic feeders were used to feed hatchery stocks, while those raised in the stream channel were fed by

hand. This subtle variation in husbandry technique could have contributed to differential survival between the two groups if seminaturally reared stocks became less cryptic due to conditioning to a surface feeding pattern.

Foraging Ability

Sosiak et al. (1979) compared the feeding habits of wild and hatchery-produced Atlantic salmon parr in a stream. They found that wild parr consumed a greater total number of food organisms and had a higher mean index of stomach fullness. In five out of six fish collections, wild fish had a more diverse species assemblage in their stomach contents than hatchery stocks. Hatchery fish tended to consume more terrestrial and winged invertebrates, while wild stocks incorporated invertebrates from the sub-stratum in their diet.

Sosiak et al. (1979) cited some earlier stream tank studies (Sosiak 1978) where- wild parr swam closer to the sub-stratum and spent more time in contact with it than hatchery parr. They speculated that wild parr foraged more successfully due to differences in use of micro habitat, a more diverse base of "search images" (Ware 1971), and better size selectivity.

Bachman (1984) found that wild brown trout (S. *trutta*) residing in a stream spent 84% of their foraging time in a "sit and wait" position at established sites with only 15% of the feed being taken off the bottom. The tendency to hold position at optimal feed locations became more entrenched with age because larger, dominant individuals could defend and hold these territories. Hatchery stocks introduced to the same stream section foraged at a number of different sites. They challenged resident fish for optimal foraging sites and often won. Unfortunately, the hatchery fish quickly abandoned the site without reaping its bioenergetic rewards. This could have contributed to lower survival of the hatchery fish due to predation (moving individuals are more visible to surface predators) and energetically inefficient feeding strategies.

Foraging Flexibility

Several studies of salmonid foraging behavior have shown contradictory results concerning the ability of hatchery-reared stocks to adapt to a natural food diet after release. Stradmeyer and Thorpe (1987) found that 65% of hatchery-reared Atlantic salmon fed on wild prey at the first feeding, and all but 3.8% of the population took wild prey within a day in a test flume. Pelleted feeds were selected at the outset but gradually they developed a preference for wild prey. The test fish took 2 to 3 times longer on average to respond to a pellet than to wild prey, but response time to capture didn't improve with experience, as observed by Ringler (1979).

Ware (197 1) found that naive, hatchery-reared rainbow trout took 4 days to acclimate to a new feed type and 11 days until response time to prey introductions stabilized (response time = time to attack and ingestion). When subjects were deprived of the specific feed for 90 days, the "search image" was lost and retraining was necessary to improve foraging responses. This information suggests that any live-feed training provided to prerelease smolts should be directed at target organisms that will provide energy-efficient forage immediately after release until they can develop alternative "search images" based on environmental feedback. It may be necessary to survey traditional postrelease foraging sites to evaluate the best organisms to use for training in any 1 year.

Findings by Paszkowski and Olla (1985) indicated that general experience with live prey can improve the overall foraging ability of hatchery-reared coho salmon smolts. Specimens which had previously been challenged to feed on Crangon with success were faster in developing capture

skills when presented with sand lances. Approximately 70% of specimens readily switched from a pelleted to a natural diet; however, 3 1.1% of the population couldn't be enticed to take either type of feed. The authors hypothesized that this behavior could be attributed to environmental sensitivity or a lack of adaptive flexibility to new forage. If this maladaptive behavior is a function of hatchery conditioning to a uniform environment, then life skills training could prove to be a valuable tool in developing flexibility in the population. An alternative viewpoint was that this portion of the population is genetically inferior and would have perished naturally had it not been for the nurturing hatchery environment. This being the case, it is unlikely that conditioning could overcome the innate weaknesses of this segment of the population.

Effect of Stock Domestication on Foraging

Ersbak and Haase (1983) found that the condition of hatchery-reared brook trout (*Salvelinus fontinalis*) diminished steadily following release into the wild when compared to wild resident stocks. Hatchery and wild stocks both fed on a wide variety of organisms, but wild stocks responded faster to quantitative changes in the forage assemblage and consequently exhibited more energetically efficient feeding and a higher mean index of stomach fullness. Hatchery stocks focused their foraging effort on a species with a similar size, shape, and color to artificial pellets instead of larger, more abundant prey. The authors noted that any inflexibility in foraging patterns resulting from hatchery conditioning could impact survival of planted fish. This implies that the hatchery diet should be diverse in shape, size, coloration, and movement.

In the same study, Ersbak and Haase (1983) observed that hatchery fish with the best condition factor at release tended to degenerate most rapidly. The authors proposed that the basal metabolism in these fish was elevated by feeding under optimal hatchery conditions which made it difficult for them to meet basic energy demands under natural conditions, ultimately resulting in attrition and death. The authors suggested that feeding programs should strive to plant hatchery stocks with a similar size distribution and condition factor to resident wild fish; the assumption being that wild fish will regulate their metabolism and growth to maximize survival with the available resource base.

Technical Approaches to Feeding Salmonids

Development of technologies to feed salmonids naturally is not a new concept. Behavioral scientists and physiologists have developed rearing vessels and subsystems that can mimic many aspects of both lotic and lentic environments for the purpose of evaluating a particular behavioral or physiological response. The challenge lies in upgrading these technologies to a production scale that can be used in raising stocks of fish at relatively high densities while developing appropriate behavioral responses to natural forage. A synthesis of the material reviewed to this point would indicate the following factors should be considered when developing new approaches to feeding hatchery stocks for release in the wild:

1. Stocks will need to develop foraging flexibility through introduction to a wide array of natural prey items to develop visual, textural, and olfactory images and to experience capturing live, moving prey. This can be done by use of live feed and/or developing artificial feeds with diverse shapes, textures, colors, and scents that will elicit a similar behavioral response in the fish.

2. Ideally, feed should be delivered below the surface in a drift form with enough current to keep it in suspension.

3. Seasonal, lunar, and diel feeding patterns for individual wild stocks will need to be evaluated. These patterns can be used to develop custom feeding programs that take into consideration spacial and temporal variations in feeding rhythm with time of year.

4. Feed will need to be delivered in low volumes and at high frequency at random locations throughout the raceway to simulate invertebrate drifting patterns and minimize territorial aggression.

5. If hatchery stocks are to develop skills in using cover and adopt a "sit and wait" pattern of territorial feeding, pond structures will need to be set up to increase the effective surface am of the system

The following sections will highlight technical approaches that we feel may have potential for altering the behavior of hatchery raised salmonids to more closely emulate the feeding behavior of their wild counterparts.

Live Food

A great deal of literature has been generated on the use of live feeds in finfish aquaculture. Technologies have been developed for the mass culture of a number of nutrient rich prey items, which are staples in the culture of marine larvae and some freshwater species. Outlining the culture and collection techniques for live feeds suitable for salmonid culture goes beyond the scope of this review, as we are primarily interested in identifying methods for delivering natural feeds and the extent to which they should be used as a part of the diet.

However, several studies indicated that salmonids offered live and pelleted feeds tend to prefer live feed despite conditioning to a formulated diet (Paszkowski and Olla 1985, Stradmeyer 1989, Stradmeyer and Thorpe 1987). In addition, several beneficial physiological responses have been attributed to the use of live feeds including increased feeding vigor, more diverse coloration, improved fin condition, and better growth rates in emergent fry (Tacon 198 1, Tonissen 1984, Surber 1935, Mathias et. al. 1982).

Holm (1987) found that Atlantic salmon alevins that began feeding on pressure shocked zooplankton had better growth rates than controls fed a mixture of live zooplankton and formulated feed until yolk sac absorption was complete. However, after yolk absorption the mixed group grew at faster rates. Fry demonstrated a preference for cladoceran species, but pressure-shocking of copepods seemed to make them more available to about 50% of the population when cladocerans were unavailable. Fifty percent of the population had no stomach contents during this period indicating a strong bias against copepods.

Stomach content analysis revealed that both groups had a similar number of zooplankton until day 75, when the group fed exclusively on zooplankton had twice the zooplankton contents of controls. These results, and improved growth rates in the control group, indicated that controls had made the transition to an artificial diet. Through stomach content analysis, the authors revealed that groups fed a dry diet supplement tended to consume zooplankton primarily at night, when formulated feeds were unavailable.

The authors recommended using live zooplankton as a first feed but switching to a mixed diet of zooplankton and formulated feed after yolk-sac absorption due to problems with growing sufficient zooplankton with a desirable species composition to meet the nutrition requirements of growing parr.

Holm and Torrissen (1987) found that at first feeding, Atlantic salmon given a frozen zooplankton supplement to their normal diet exhibited a dramatic drop in growth rate and reduction in production of proteolytic enzymes. The feeding response in zooplankton-fed groups was more vigorous than that of the control group. The authors speculated that increased energy consumption associated with the vigorous feeding behavior and a reduced coefficient of digestibility caused by high chitin levels in the freeze-dried feed caused the depression in growth.

Studies by de la Noue and Choubert. (1985) demonstrated that feeds created from freeze-dried zooplankton had a lower digestibility coefficient in rainbow trout than a reference diet based primarily on fish meals. They speculated that high chitin levels were a major contributing factor to the reduced digestibility. Beck and Posten (1980) studying silverside larvae suggested that processing of live feeds caused oxidation and loss of valuable micro ingredients (i.e., exogenous proteolytic enzymes, long-chain fatty acids, steroids, and carotenoids) that facilitate digestion and assimilation of prey when consumed live. This could explain the dramatic difference in growth rates and survival between fish fed a live diet and one that was freeze dried. Torrensen (1984) found that Atlantic salmon parr fed a diet supplemented with cantaxanthin and astaxanthin had improved growth rates, supporting the hypothesis that carotenoids play a role in feed assimilation. These studies indicate that current processing methods are inadequate for maintaining the nutrient quality of live feeds.

Holm and Moller (1984) found that Atlantic salmon yearlings could survive adequately on a diet of zooplankton, provided there were high enough prey densities and the species composition was adequate. The highest growth rates attained were 0.93 mm/day. Stomach analysis revealed preferences for large daphnids.

Atlantic salmon parr raised in net-pens by Pepper et al. (1987) grew best on a mixed diet of zooplankton and artificial pellets. Fry raised in this manner grew at a maximum rate of 3.09%/day. Stomach content analysis revealed that subjects fed a mixed natural/artificial diet had more zooplankton in their systems than controls fed exclusively on zooplankton. Holm (1986) found a strong correlation between current velocity and feeding success of Atlantic salmon fry. If currents were too slow through the rearing structure, zooplankton tended to aggregate in patches and fry would starve rather than extract individual zooplanktors from the mass. When the flow was increased, the patch dispersed and alevins began feeding.

Surber (1935) found that brook trout raised on a diet of amphipods (*Gammarus fasciatus*) required 6.05 g (wet weight) of live feed to produce a gram of growth: analysis revealed 58,000 amphipods per kg on average. The author noted that fish used in the experiments developed more brilliant coloration than fish raised on a standard hatchery ration. Mathias et al. (1982) found that *Gammarus lacustris* could be harvested at an annual rate of 500 to 1,000 kg/hectare from a northern lake system, depending on the degree of productivity. Cost-benefit analysis revealed a cost of $0.80/kg to collect *Gammarus* using set nets. Large aggregations of *Gammarus* could only be found in spring and fall, thus provision of a year round supply would be difficult. The author estimated 16,000 individuals/kg of *Gammarus* collected, and noted improved survival and better external coloration in rainbow trout raised on the live diet.

In summary, the data suggests that live feeds can be beneficially incorporated into the diet of swimup fry and as a supplement to the formulated diet for growout. It would appear that parr will readily accept live feed while being fed a formulated diet, thus it should be feasible to introduce novel prey items throughout the hatchery cycle for sensory training purposes and to develop skills in capturing live prey. The exclusive use of zooplankton as a feed source would be

technically problematic at best, due to the sheer numbers that would be required to promote positive growth in the rearing space typically found in a hatchery facility (Holm 1987).

Live Feed Delivery Systems

Potential live-feed delivery systems range in complexity from simple systems that pour zooplankton/water mixtures through a submerged tube to sophisticated systems that incorporate holding tanks with built-in life support systems. Generally, a system needs an aerated holding tank to keep organisms aerated and in suspension, a timer mechanism for dispensing calibrated amounts of feed, and tubing to carry the feed/water mixture to the fish-rearing structure. If live feeds are used as a first feed for hatchery-reared salmonids, it will be necessary to deliver and distribute feed through Heath trays or rearing baskets in troughs.

A number of systems have been developed that can fit into this scheme. Prentice (1975) developed an automated feeding system that consisted of an aerated holding tank with a depth-sensing rod to deliver *Artemia* to post-land spot prawns. Feed was drawn from the holding tank at timed intervals for distribution to rearing structures via tygon tubing. Individual portions to a specific pond were regulated by hose clamps attached to the feeding tube. Between feeding cycles, the lines were flushed by passing water from a header tower to the individual rearing units. A system of check valves prevented flooding or dilution of the holding-tank water supply by the header tank. A ball valve on the outflow from the feed delivery pump was used to regulate water pressure. This system was designed for laboratory-scale operations but could easily be upgraded for mass culture operations.

Theis and Howey (198 1) developed a gravity operated system for delivering live feeds to larval walleye and American shad. Feed was moved by gravity from an acrylic holding tank to a solenoid valve via a latex or tygon tube. At timed intervals, the solenoid valve opened to release a feed/water mixture into the rearing pond The timing mechanism consisted of three separate units: a 24 hour timer to control the length of the daily feeding, an interval timer to control the length of times between feedings (range every 2 minutes to 4 hours), and a time-interval relay to control the length of each feeding (0.6 to 60 seconds).

Microprocessor-based timers are now available that can perform all these functions from one unit (Hortimic Inc., Finland). Live feeds were kept in suspension and aerated by an air stone in the holding tank. However, a separate discharge solenoid was required for each rearing unit.

Anderson and Smith (197 1) developed a brine shrimp feeder requiring no power source using a siphon effect. Water was drip fed from a constant-flow siphon into a sealed, aerated, mason jar through a funnel. When the water level in the jar crested above the level of discharge, it triggered a siphon action: The brine shrimp/water mixture discharged until the mixture reached the inlet portion of the discharge line inside the mason jar. At this point air entered the line, breaking the siphon, and the cycle repeated itself when the water level increased to a preadjusted height in the funnel.

Time intervals between feedings were controlled by increasing or decreasing the rate of filling the mason jar. The volume of organisms discharged at individual feedings was regulated by adjusting the depth of the discharge line in the mason jar. The system, as it was originally designed, would have limited applicability in production aquaculture. However, the underlying operating principle could be used to design a system with multiple discharge points suitable for large-scale dispersion of live feeds.

Modifications to Formulated Feeds

The use of live feeds in the diet would require that stocks be weaned to an artificial feed for growout. Since salmonids appear to have an innate preference for live feeds, it would therefore be prudent to expect food-acceptance problems with pelleted diets to arise during the initial weaning period and possibly after each instance when live feeds are offered One possible approach to getting around this problem would be to modify the physical attributes of the formulated feeds to improve their sensory characteristics.

The few studies that have been carried out to assess the effects of artificial feed shape, size, color, smell, and taste on behavior indicate that significant gains can be achieved in both fish growth and feed acceptance by manipulating these sensory-triggering components of the diet. If formulated diets can be created that elicit a natural-type response from hatchery stocks, it may be possible to achieve adequate sensory training in stocks without the use of live feeds, thus avoiding the expense of maintaining labor intensive, live-feed culture facilities at hatchery sites.

Feed color preference--Jakobsen et al. (1987) found that Atlantic salmon fed a two-colored diet had faster growth rates 'and narrower population size distributions than controls raised on a standard diet. The authors proposed that subjects became visually confused when presented with a large mass of homogenous pellets and the two-colored diet reduced confusion by enabling individuals to focus on one prey item. The authors suggested that factors such as swarm size and visual density increase confusion. They noted that changing the visual characteristics and density of feeds could improve feed distribution, enhancing production in intensive culture situations.

Ginetze and Larkin (1973) studied the effects of color on the feeding behavior of rainbow trout in an artificial stream. The trout demonstrated a significant preference for different colors and even for specific combinations of colors, depending on background color and light intensity. For instance, feeding combinations of yellow and black eggs led to higher feed consumption of both feed types, a behavior that agreed with Jakobsen's (et al. 1987) findings. Interestingly, all colors were consumed at higher levels in the presence of yellow feed, regardless of light conditions. Color contrast and light intensity appeared to be the most important factors influencing color selection, although some colors were preferred regardless of background coloration. With a blue-green background, egg consumption was highest for blue eggs followed by red, black, orange, brown, yellow and green.

In experiments where prey was viewed against a natural transparent background, Dendrinos et al. (1984) found that dyeing *Artemia* with black food coloring increased larval dover sole feeding efficiency from 20 to 60%. Subsequent trials with other colors highlighted the following color preference ranking in descending order: black, red, pink, yellow, blue, and control (transparent). The authors speculated prey contrast with the background was the major factor contributing to the remarkable improvement in performance.

Wolf (1953) reported that rainbow trout fed vigorously on pellets dyed a red color, requiring no special treatment during weaning from meat diets. Reactions to green, blue, yellow, and uncolored diets were passive, with much of the feed being wasted. The diet was dyed using 1/2 oz. FD&C Amaranth #2 powder in 6 L of water for every 45.4 kg feed. The results from field trials were promising enough to encourage feed manufacturers to offer their services in producing the diet. Walleye culturist have observed that larvae tend to prefer orange and red colored feed particles over those of the regular diet, but no conclusive studies have been carried out to confirm these observations (Nickum 1986).

Evaluation of this limited data suggests that prey identification and selection can be improved by presenting mixed-color diets in colors that strongly contrast that of the rearing environment.

Feed texture preference-Stradmeyer et al. (1988) evaluated the response of **hatchery-**reared Atlantic salmon to the textures of feeds. They found that long, thin, soft pellets were consumed twice as often as hard, long, thin pellets. Stradmeyer et al. (1988) cited unpublished data indicating that juvenile Atlantic salmon **challenged** to feed on live prey and long, soft, thin pellets ingested twice as many live prey. Texture and taste were considered the main differences influencing choice.

Feed shape preference--Stradmeyer et al. (1988) evaluated the feeding response of hatchery-reared Atlantic salmon juveniles to alternatively shaped and textured feeds. Ingestion of long, thin pellets was four times that of cylindrical pellets. Subjects responded well to a long fat pellet up to capture, but 70% of these pellets were rejected before ingestion, as they were to large to swallow. The test fish did exhibit a learning effect as they attacked fewer large pellets as the experiment progressed. **While** not much information is available on the feeding response of salmonids to alternatively shaped feeds, the literature for teleosts in general suggests that prey shape plays a critical role in prey identification and capture (Hyatt 1971, Knights 1985).

Feed odor and taste preference--There is evidence to suggest that smell and taste enhancers can be used to improve the location of feed and influence acceptance of formulated diets (Hughs 1991, **Loveshin** and Rushing 1989). It appears that gustatory stimulants are **species-**specific and quite complex, thus much of the work to date has focused on identifying specific compounds that enhance flavor or elicit a feeding response **(Atema** 1980, **Mackie &** Mitchell 1985).

Mearns et al. (1987) studiedthe effects of chemical fractions and extracts from shrimp flesh on the feeding response of adult rainbow trout and Atlantic salmon **parr.** The Atlantic salmon were more sensitive to the chemical composition of the diet: low levels of ingestion were observed for all treatments except those containing pure extracts from the shrimp.

These results were somewhat inconclusive, as the rainbow trout developed a taste for the agar-feed base and consumed all fractions with equal enthusiasm The author speculated that amino acids acted as chemoattractants to assist with orientation and enhance appetite, while **water-**soluble proteins were more important for inducing swallowing of the diet.

Hughs (1991) studied the response of first feeding chinook salmon to chemical attractants. The data indicated that feeding responses increased with the addition of glycine to the diet at a level of 1%. Preliminary results from subsequent trials indicate that chinook salmon's positive response to glycine decreases with age. A shift in preferences to **proline** and anhydrous betaine with aging was observed. **Proline** appears to be an active feed stimulant throughout the lifecycle (Steven Hughs, pers. .commun.).

Work by **Rottiers** and Lemm (1985) indicated **that** walleye can be attracted to a feeding station and exhibit an exploratory feeding response by simply placing water from ***Daphnia*** or ***Artemia*** culture tanks in the feeding chamber. If this is the case, we may be able to bypass placing feed attractants or chemical scents for developing "olfactory imprints" in the formulated diet. Cultures of target organisms could be raised on a small scale, with the effluent from these culture tanks being used to provide olfactory training to hatchery stocks. At regular intervals, invertebrate

prey could be "cropped" and fed to fish to create a "visual imprint" (Ware 197 1) to complement the olfactory imprint.

Atema (1980) suggested developing food-odor associations during the hatchery phase of ocean ranching programs, but cautioned that **reenforcement** by repeated presentation of the odor was required to avoid extinguishing the imprint.

Extrusion Technology

Extrusion technology has developed to a point where high-protein diets can be extruded in a number of different sixes, shapes, and densities using the same basic ingredients as in pellet presses, while maintaining similar, if not better, nutrient and digestibility characteristics. The **high-**pressure cooking process allows for gelatinization of carbohydrates and denaturing of proteins into basic amino acids without destroying their nutrient qualities. This process allows manufacturers to use a wide variety of protein sources with similar amino acid **profiles** to produce a uniform-quality feed using least cost formulation strategies.

Gelatinization of the starch component of the diet creates a strong binder that resists both water breakdown after emersion and physical breakdown during shipping and storage. The use of extrusion technology for production of fish and shrimp feed has evolved over the last 30 years, with most development work being done by private manufacturers looking to capture a portion of the aquaculture market.

Therefore, exact feed formulations and manufacturing protocols are closely guarded trade secrets, and the most comprehensive processing information is available through extruder manufacturers. In conversations with several feed manufacturers, it would appear that very little research effort has been expended to fully utilize the capabilities of modem extrusion technology (as seen in the pet food industry) to enhance the sensory stimulating qualities of formulated fish diets. **Extruders** and the associated drying equipment are expensive, thus most machines are dedicated to ongoing feed manufacturing.

Use of this equipment is expensive for pilot-scale development work as minimum run sixes are approximately 900 kg for large machines and production must be halted to run the trial. The current product line offered by feed companies meets the needs of most production aquaculture operations, where behavioral training of hatchery stocks to emulate the actions of wild stocks is not an important production criteria. There is little economic incentive for feed manufacturers to develop these innovative feeds, as "natural" rearing practices for salmonids have not been sufficiently developed and tested for incorporation in production level hatcheries.

Feed color--Jakobsen et al. (1987) coated feed pellets with diethyl ether to create a thin oil layer by drawing lipids inside the pellet to the surface. This oil layer was coated with 2% **curcumin** El00 to give pellets a light yellow-green color. The pellets were then dried for 24 hours at 25°C.

Wenger **Inc.'s** (Sabetha, KS) Feed Extrusion Technical Center has produced soft, moist fish-shaped cat feeds in a variety of colors. The diets were produced with up to 40% protein and **20-25%** moisture. The director of the lab indicated that difficulties might be encountered in creating bright colored feeds due to both the dark pigmentation of fish-based protein sources traditionally used in fish feeds and the natural darkening of feed from cooking in the extrusion process.

FD&C certified colors are used extensively in the production of dry extruded pet foods. These dyes are generally applied at a rate of 0.010 to 0.030% of the overall diet and added to the dry mix or dissolved in liquid, which in turn is injected into the extruder. The high moisture content in semi-moist feeds take the dyes well. The least expensive dyes are powdered forms that are water soluble. If leaching is a problem, oil-soluble dyes are available at a higher cost (Noonan 1968). During the extrusion process, 10 to 15% of the dye is lost, thus adding extra dye is recommended to attain the desired color.

Feed attractants--Shrimp feed manufacturers regularly incorporate feed attractants into pelleted diets-to increase palatability and to attract shrimp to the feed in murky, brackish-water ponds. These attractants are generally water soluble and are leached slowly from the pellet to create a lingering olfactory trail. These attractants are either incorporated into the dry ingredients before extrusion or sprayed on after drying (Hyme Garcia, Production Manager, Moore-Clark Co., La Conner, WA, pers. commun.).

Feed texture--A number of feed companies have developed semi-moist or semidry pelleted feeds that have improved the feed acceptance levels in some cultured species. This product is extruded in a similar manner to floating hard pellets, but the moisture content in the mixing chamber is raised initially to 30-32%. After drying, the product has a moisture content of approximately 25%. Spoilage is minimized by addition of preservatives including propylene glycol, potassium sorbate, salts, and acids. The mixture of these ingredients is different for each species raised dependent on taste preferences (i.e., dogs prefer a sweet flavor while cats prefer acidic flavors, Keams 1988). This product is physically durable and withstands normal packaging and storage.

One option being considered for feed development is to produce a product that is pliable and would "move" when placed in a water current, much like natural prey. The production of a gelatinous pellet could be difficult due to the high percentage of processed animal proteins traditionally found in aquatic feeds and to the restrictions on overall carbohydrate content in salmonid diets. It may be possible to produce a semi-flexible diet through use of binders such as sodium alginate, and carrageenan to augment the gelatinizing properties of carbohydrates (John Krehbiel, Wenger Inc., Sabetha, KS, pers. commun.).

Vegetable proteins can contribute significantly to the development of texture in the final extruded product, as they develop elastic properties when denatured through the extrusion process. These strands of elastic protein can be manipulated to produce a "muscle like" texture. This characteristic can be manipulated with emulsifiers and PH adjustments to control the "chewyness" of the final product (Hauk and Nielsen 1983).

Previous attempts to incorporate vegetable protein into the diets of Pacific salmon have resulted in reduced growth rates and poor performance compared with animal protein substitutes (Fowler 1980). However, manufacturers claim that the moist-heat extrusion process improves the availability of amino acids and destroys many anti-nutrient compounds found in vegetable proteins (i.e., 95-99% of trypsin inhibitors are denatured) while extruding the feed (Hauk and Nielsen 1983). Addition of minor quantities (15%) of vegetable proteins doesn't significantly alter feeding performance in Pacific salmon (Fowler 1980) but could contribute to improved textures for heat-extruded feeds.

Feed shape--The production of intricately shaped products or very small pellets requires the use of a twin screw extruder with multiple dies to regulate passage and shaping of the extrudate to ensure consistent product quality (Hauk and Nielsen 1983). Twin screw extruders are

recommended for applications where of the formulation consists of more than 40% protein or the total internal fat content exceeds 15%. Feed manufacturers using single screw extruders have manipulated the feed formulation by lowering the internal fat content to 8% and increasing the protein content to produce a commercially acceptable product using less costly machinery. Additional dietary fat is sprayed onto the diet to achieve the desired overall fat content (John Krehbiel, Wenger Inc., Sabetha, KS, pers. commun.).

The smallest size feed that can be produced in distinguishable shapes is in the range of 2.5-3.0 mm with ingredients typically used in fish feeds. When producing shaped products, exuuders will yield approximately 50% less product in a given time frame due to limitations on the number of holes that can be placed in the die when compared to pelleting (Frank Hertzo, Wenger Inc., Sabetha, KS, pers. commun.).

Feed density--Feeds delivered by a water-based medium will need to be kept in suspension when delivered below the surface, thus it is important to have control over the density of the formulated feed. When producing floating feeds, a density range of 320-400 g/L is desirable. Typically floating feeds are cooked with 24-27% moisture to a temperature ranging from 125 to 138°C inside the die of the extruder head. Feed is extruded at a pressure of 34-37 atmospheres through the die. Upon exiting, the feed expands by 125- 150% as water in the formulation vaporizes and expands. The diet is then dried and cooled to a moisture level of 9-l 1% (Kearns 1988, Hauk and Nielsen 1983).

Sinking feeds can be produced using a similar extruder configuration to that for floating feeds. The density of these feeds are typically in the range of 400400 g/L. Feed is run through the extruder at a moisture level of 30-32% at a slower speed to control temperature buildup to a maximum of 120°C behind the extruder die. This formulation is passed through a larger die at a pressure of 26-30 atmospheres where it expands 10- 15% . The feed is then dried to a final moisture content of 10-12% (Kearns 1988, Hauk and Nielsen 1983). Density of the final product can be varied, from almost neutral to strongly negatively buoyant, by controlling the rate of drying and final moisture content (Bioproducts Inc., Warrenton, OR, pers. commun.). Subtle variations in production procedures can produce feeds in a wide range of densities using extrusion technology.

Hydromechanical Feeding Systems

A review of the methodologies from the general aquaculture literature reveals that automated feeding systems for production culture of salmonids have largely evolved out of the poultry and livestock industry. The adoption of these systems was a logical extension of the incorporation of pelleted feed into aquaculture programs. Major adaptations to these systems have been to create mechanisms to spread feeds evenly over large surface areas and creation of pellets that will sink or float dependent on species requirements.

These systems are adequate for production operations where fish are raised until harvest in the sheltered hatchery environment. Problems arise when stocks raised for supplementation or ranching programs are required to leave this sheltered environment: fish must fend for themselves in the natural environment and secure adequate forage without previous experience with prey items. Behavioral scientists have recognized the importance of feed presentation to the development of natural foraging patterns and have built systems that emulate invertebrate drift patterns. As the feeding systems have only been a small component of most project objectives in past studies, little descriptive data was available on the design or function of these systems.

Laboratory studies--Wankowski (1979) injected feed pellets below the surface and allowed a flume-generated current to carry pellets downstream to the subjects. Current velocity was adjustable from 0.10 to 0.22 m/s. Stradmeyer et al. (1988) released feed into a funnel fed by a continuous supply of water. The pellet/water mixture was carried below the surface by a plastic tube about 50 cm ahead of the feeding station, and current was regulated by a series of baffles. Stradmeyer and Thorpe (1987) conditioned stocks for experiments in a circular flow tank with feed delivered to the bottom of the tank with the water supply. Feed was carried to the subjects in a current equivalent to 3 fish body lengths per second.

Fausch and White (1983) at the University of Michigan developed a Sinuous Stream Aquarium that used an airlift pump to generate current. They released live *Daphnia* into the riffle zone and allowed generated currents to carry prey downstream in a simulated invertebrate drift. The authors noted that test fish competed for optimal foraging locations as they would in nature, indicating the simulation was a success. The factors they attributed to this success included the shape of the rearing vessel, current velocity, and configuration of the substrate.

Irvine and Northcote (1983) artificially generated current in a stream tank using submerged sump type pumps. Feed was delivered into the current by pouring a water/feed mixture into a funnel. Paszkowski and Olla (1985) developed a feeding tube to present both live and pelleted feed into the test tank. Water and feed were poured into the feed tube simultaneously resulting in feed entering the tank at mid-level. A continuous current, generated by pumped estuarine water, kept the feed in suspension for several minutes.

Bugert and Bjornn (1991) tested habitat utilization by coho salmon and steelhead (0. *mykiss)* in the presence of a predator using a simulated stream section in the laboratory. Feed was delivered from a hopper into a water-fed funnel where it was mixed and then flowed down a tube to a perforated pipe buried in the riffle gravel, with one unit placed in each riffle zone. Current was generated by a paddle wheel at the head of the stream system. The distribution of feed appeared to have been adequate, as no aggregations around the feeding tube were observed.

A review of these innovative but simple subsurface feeding systems indicate that two factors are important to establishing and invertebrate drift: there must be an adequate current velocity, and feed should be neutrally buoyant or slightly positive to keep it in suspension.

Production-scale subsurface feeders--There were several cases cited in the literature we reviewed where a production facility has adopted a program of delivering feed below the surface or used a water-based delivery system. Glenny (1932) developed a "water dissemination system" for feeding fish in earthen ponds. The system basically consisted of a water line that fed a . pond which was attached to a filtration box filled with ground feed As water passed through the system, it diluted the mash and carried small particles out to a series of submerged perforated pipes for distribution to the stocks. The brief description of the system's function indicated it worked well. Ghittino (1972) illustrated an automated feeding system developed in Alkarleo, Sweden, that operated by water flow. Unfortunately, no descriptive material was provided on the system's operation.

Ruohonen (1986) described a computer-regulated feeding system that delivered feed to raceways in a water stream discharge at the water surface that resembled surface-drifting patterns of feed in a stream. The systems computer software calculated daily feed requirements based on population size, using a bioenergetic model that considered standard growth rates and input from waterquality sensors (temperature and oxygen). Feed was dispensed in small portions from 100 to 1,000 times daily depending on programming.

Tests of system operation indicated that 60 feedings a day were optimal for Atlantic salmon presmolts. Size variation in test groups narrowed with increased feeding frequency, indicating better distribution of feed in Atlantic salmon. Atlantic salmon fry raised using the system had 50% greater overall growth than controls fed by hand. Feed conversion ratios were also better in groups fed with the system (Ruohonen 1986). The system is now marketed by Hortimic LTD., and used extensively in Atlantic salmon hatcheries throughout Norway, Sweden, Finland, and Canada (Kari Ruohonen, pers. commun.).

The Ole Molaug Company has developed a subsurface feeding system that can deliver feed in a water or air flow to a depth of 40 feet. The heart of the system is a pellet pump that gently mixes water and feed together in a fast-flowing stream and delivers it to net-pens via a computer modulated distribution manifold (Fish Farming International 1981). Minnesota Aquafarms (Chisolm, MN 557 19) developed a modified version of this system to deliver floating pellets to chinook salmon in 40-foot-deep net-pens. Apparently, the stocks feed low in the water column in a calm manner. When fish are satiated, excess feed floats to the surface, alerting staff to adjust ration levels (Richard Noble, Minnesota Aquafarms, Chisolm, MN 557 19, pers. commun.).

Integrated Aqua Systems (IAS) Products, Ltd. (Box 52010, North Vancouver, B.C. V7J 3T2), has developed a water-based feed delivery system primarily designed to mix water and pellets in a venturi pump and deliver it in a spray at the water surface. The water-based delivery breaks surface tension, allowing feed to sink easily into the water column. This system should also function as a subsurface delivery system, provided the depth of the discharge port doesn't exceed 2-3 ft. Apparently, at greater depths the back pressure on the venturi will obstruct venturi action (Engineers, IAS Industries, pers. commun.).

Remote Site Feeders--Several innovative feeders have been developed that require no power and that might have applications for remote site operations. Zemora (1985) developed a water-activated feed dispenser that could release dry feed at intervals determined by the time it takes to fill a counterweight vessel. When the weight of the water exceeded that of the counterweight the arm dropped, emptying a volume of feed into the pond and emptying the water chamber to reset the feeder. Time interval between feedings was adjusted by increasing or decreasing water discharge into the chamber. The author indicated that the system was simple and inexpensive to construct and operate.

Baldwin (1983) developed a feed-delivery system capable of dispensing standard volumes of feed on a 24-hour basis at predetermined intervals. As water dropped into a calibrated chamber, a weighted float line turned a carousel with built-in feed chambers. The individual chambers opened as the carousel moved past a trigger that slid open a release gate. The system operated successfully for several seasons of black molly culture, but needed to be reset daily.

Both these systems could be set up over the water-intake structure in an acclimation pond to dispense feed directly into the water current. With multiple intake pipes to the rearing structure, feed distribution could be further refined. In locations where power supply is not an issue, most commercially available surface feeders with hoppers can be adapted to deliver feed into a naturally or artificially generated current.

Summary--According to the literature reviewed, it appears that no one system currently available or under development can meet all the specialized requirements of the NATURES program. However, certain components from currently available systems could be incorporated into a system, such as the pellet pump produced by Ole Molaug. The major limiting factor precluding the use of available systems is that most are designed to deliver feed from a limited

number of discharge points, and raceway culture generally requires multiple discharge points to ensure even feed distribution.

Recommendations

A review of the literature indicates that development of foraging behavior in juvenile salmonids is brought about by a complex interaction of habitat selection, availability of prey, inter- and intra-specific competition for forage and cover, innate and acquired feeding rhythms, age of fish, and physiological requirements. The ultimate survival of the individual is largely dependent on how it balances these factors to ensure adequate and efficient energy intake while avoiding predation. Selection pressures in the stream environment favor those individuals that adopt a "sit and wait" posture: to stay behind cover awaiting the delivery of invertebrate drift. This behavior is energy efficient and minimizes the chance of an encounter with predators.

The conventional hatchery environment limits development opportunities for juvenile salmonids reared for supplementation. For instance, in the wild, juvenile salmonids are segregated by size in the stream environment as a function of their ability to maintain position in swift currents. Larger fish prefer deeper, swifter pools where foraging opportunities are better. This creates a natural social barrier between large predatory fish and juveniles. Hatchery fish are accustomed to deep, slow-moving rearing vessels, and after release, they gravitate to deep pools where they must use excessive energy to hold position against the swift current and increase their exposure to large predatory fish.

The NATURES project seeks to identify characteristics of the hatchery environment that predispose stocks to failure in the wild, and to develop new and innovative hatchery technologies that promote the development of behavior that is adaptive for survival in the wild. The Abernathy Salmon Culture Technology Center has investigated development of a feeding system that promotes natural foraging behavior in salmon. A comparison of feeding options available to wild-reared fish and hatchery-reared fish indicates that hatchery procedures have been over-simplified, limiting the development opportunities for hatchery parr, and in some cases promoting maladaptive behavior for functioning in the wild

Examples of maladaptive behavior include: 1) training of stocks to feed only during daytime working hours even though data indicate that wild salmonids are crepuscular feeders (dawn and dusk feeders) for most of the year, with at least a portion of the diet being taken nocturnally, 2) reduction in foraging flexibility caused by conditioning to one feed type, and 3) in programs where fish are fed by hand, stocks are conditioned to approach large moving objects at the surface, thus predisposing them to attack by surface predators after release.

Based on reviews of the literature, we conclude that a new model for fish feeding in supplementation hatcheries is needed and that this model must address species-specific developmental, behavioral, and physiological requirements. Factors that should be taken into consideration include:

1. Diel, lunar, and seasonal variations in feeding patterns that follow the predictable succession patterns of prey availability in the wild. The literature for Atlantic salmon indicates that foraging behavior changes considerably through seasons with respect to prey preference, feeding times,-feeding intensity, and position in the water column.

2. Space requirements for development of foraging behavior that emulate the natural pattern of territorial feeding and use of cover.

3. Development of age-specific feed formulations that elicit natural foraging responses and that meet nutrient requirements. This feeding protocol may require the use of live feeds, formulated diets, or a combination of the two to achieve the desired response.

4. Feed for juvenile salmonids should be delivered in a drift form, resembling invertebrate drifting patterns.

5. Feeds should be diverse in color, scent, texture, shape, and size to allow for development of prey selection skills, thus improving foraging flexibility.

We conclude that the first objectives in developing this model should be 1) to build an automated subsurface feeding system with sufficient flexibility to emulate the natural invertebrate drift patterns found in streams, and 2) to develop formulated diets that function in this system A third objective in creating these new formulated diets should be to improve presentation by mimicking some of the sensory-stimulating qualities of live, natural feeds.

Reports of studies carried out in these areas with Atlantic salmon and rainbow trout indicate that there is considerable promise for improving production by following this line of study. With development of a more flexible feeding system and diets with improved-sensory stimulating qualities, we will have the tools needed to refine and customize feeding procedures for individual species that take into consideration the five factors outlined above. Some of the work on these factors can be carried out concurrently with the feeding project, while others will require further review of the literature and field research on wild foraging behavior for individual stocks.

These modifications to feeding technique are likely to improve the postrelease survival of hatchery-reared stocks by improving foraging flexibility. Development of improved foraging ability through natural feeding techniques, complemented by methodologies being developed in other areas of NATURES research should contribute to production of a smolt that reacts more naturally to its environment after release from the hatchery. This adaptability should contribute to improved postrelease survival to adulthood and ultimately, to restoration of depleted salmon runs in the Columbia River Basin.

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